THE PHILIPPINE
JOURNAL OF SCIENCE

VOL. 43 NOVEMBER, 1930 No. 3

# STUDIES ON THE DEVELOPMENT OF THE SUGAR-CANE PLANT IN THE PHILIPPINES

## ROOT AND SHOOT DEVELOPMENT OF M–1900 [1]

By MANUEL L. ROXAS and MANUEL VILLANO
*Of the College of Agriculture, University of the Philippines, Los Baños*

FIVE PLATES AND THIRTEEN TEXT FIGURES

## INTRODUCTION

The studies of the root and shoot development of the M–1900 variety of sugar cane reported in this paper were planned by the senior author and made by the junior author at the experiment station in Canlubang, Laguna Province, Luzon. Therefore, responsibility for the ideas given in this report rests mainly on the former and responsibility for the accuracy of the results is laid at the door of the latter.

Following the example of investigators in other countries in the study of the root development of the sugar cane and other plants, we started these studies in the Philippines, believing that a clear understanding of the formation and behavior of roots of the cane, under our diversified soil conditions, will dictate the best fertilizing and cultivation methods for our fields.

[1] Preliminary report 1. The results reported in this paper were obtained from the thesis presented by Manuel Villano as a part of the requirements for the degree of Bachelor of Science in sugar technology at the College of Agriculture, University of the Philippines. They are published with the permission of the Dean of the College of Agriculture.

## ROOT STUDIES IN OTHER COUNTRIES

The excellent publications of Weaver and his coworkers (1–5) have furnished the background for the root studies of sugar cane conducted during the past years in Hawaii. An excellent summary of the general knowledge of the development of roots of the cane and other plants is given by Wolters. (6) However, since all discussion of the root studies in Hawaii are treated with the Hawaiian agricultural practice in mind, we will select from the original sources of information the high lights of the present knowledge of root development, having in mind their possible bearing on the practices that have resulted empirically from the different climatic and soil conditions in the Philippines.

1. It appears to be a definite conclusion that heavy root weights are correlated with heavy cane tonnage. (6)

2. Although absorption of nutrients by roots takes place throughout the whole extent of the root system, it occurs most actively in the younger and usually deeper parts. Root hairs, the organs of absorption of the roots, are limited to the younger portion except the zones of division and elongation. However, the abundance of root hairs is dependent on water content and air supply; there are very few in very wet soil. Roots die in stagnant water. (5)

3. While frequent light showers during the planting season or too early application of irrigation water will induce the formation of shallow root systems, a period of drought or the proper cultivation method will stimulate the formation of deep roots. The formation of roots is greatly governed by the distribution of moisture in the soil. (4)

4. The old idea of chemotropism, according to which, roots will deliberately follow a rich source of nutrients, must be modified in the light of the experiments of Weller. (7) Roots develop according to a hereditary habit, which may fix their form and direction. The expansion of the roots, however, depends on the conditions immediately surrounding the roots. When a root stem or branch, in its progressive development, encounters a layer of rich moist soil, extensive branching and root-hair formation will occur mostly in this layer, though the effect will not be limited to that layer, but increases will be stimulated throughout the whole root system of the cane.

5. However, Weaver demonstrated that in the case of the plants used by him, the penetration of the roots to the lower levels is retarded by such a rich layer, if diffusion of nutrients from one layer to another is prevented. Quoting from him,

> In every case where roots came in contact with a fertilized layer they not only developed much more abundantly and branched more profusely, but such a layer apparently retarded normal penetration into the soil below. Thus, it seems that the depth at which the fertilizer is placed in field practice would considerably affect root position and development. Fertilizing the surface layers of soil in regions where these have very little or no available water during periods of drought, would appear to be distinctly detrimental to normal crop production.

6. Contrary to statements made recently by some investigators, the deep layers of soil are just as important as the surface layers to the life even of those plants with 70 per cent of their root system in the 8-inch topmost layer. To quote from Weaver(3) again,

> * * * since the roots of crop plants are found to penetrate just as deep or even deeper under field conditions as in the containers used in these [Weaver's] plot experiments and since their development in every respect has been found to be identical, we must conclude that the deeper soils are not only suited to plant life, but that they play an exceedingly important part in the life of the plant and deserve careful consideration in a study of crop production.

However, the results of our present work are not in line with the experience of Weaver, as root penetration in our box experiment is much deeper than in the field experiment.

7. *Distribution of the roots of the sugar-cane plant.*—The investigations carried out by the Hawaiian Sugar Planters' Experiment Station, in which the method of excavation proposed by Lee(8) was used, have shown that in mature canes, "about 70 per cent or more of the roots were found in the topmost 8 inches of soil."(6)

8. While it has been demonstrated that a very large proportion of the root masses of the cane is in the topmost 8 inches of soil, no experiments similar to those conducted by Weaver on the rate of absorption by roots in different levels have been done with the cane, so that nothing definite is known with regard to the relative physiological importance of the 70 per cent in the topmost 8 inches, and the 30 per cent in the lower levels. Until

this relative physiological value is known, no one will be in a position to form a well-balanced judgment of the function of the different parts of the root system of the cane, or to say that soil levels where only very small masses of root are found are of little value to the crop and may be left out of soil studies. On the contrary, their nature may, for all we know, determine the success or failure of a crop under extreme conditions of drought and rainfall.

9. A recent publication by T. S. Venkatraman and R. Thomas(9) gives the results of studies of sugar-cane roots at various stages of growth. From the point of view of root distribution, perhaps the most interesting habit of the cane roots discovered by these authors is the difference in the aërotropism of the roots of different varieties. For instance the sett roots of Striped Cheribon (a noble cane) are more aërotropic than those of Katha, an Indian cane not quite intolerant to water logging. This aërotropism of the roots of canes will help explain the tendency to have more roots towards the surface. The following summary and conclusion are from the article of Venkatraman and Thomas:

> During the germination or sprouting of sugarcane setts, the development of roots from the dormant root eyes is one of the first activities.
>
> The roots thus developed from setts have been styled "sett" roots in contrast to "shoot" roots which are developed later from the shoots.
>
> There is no correlation between sett root production and the sprouting of buds. For the full development of the bud into a shoot, however, sett roots are essential.
>
> Interesting differences exist between cane varieties in the number, length and functioning period of sett roots produced during germination. In certain cases only a portion of the root eyes produce roots, the rest remaining dormant till a need arises. This is considered to be a definite and valuable provision against possible adverse conditions during the later stages of growth.
>
> Irrigation with saline water is harmful to sett root development and should be avoided.
>
> In most canes, sett roots die after a time and, subsequent to this, the plants are dependent on 'shoot' roots developed later from the young growing shoots.
>
> The rate of growth of the above ground portion of the cane plant is positively correlated with the growth vigour of shoot roots and the study of these roots thus becomes a matter of great importance to the cane grower.

During the adult stage of the cane plant there is almost a continuous development of new roots, resulting in a constantly changing root system which readily adjusts itself to changes in the environment.

If the cane is prevented from thus developing new roots, it gradually loses vigour and dies.

It is desirable to work out for each variety its typical root system and find out exactly where (in the soil) and when the series of successive new roots are developed. Such knowledge would materially help in indicating beforehand the conditions under which the variety is likely to do its best. It would further be of great use in guiding manurial and cultural operations with the maximum advantage to the growing crop.

Sett roots differ from shoot roots in certain respects, the generally greater growth vigour of the latter being the most important difference.

The observed differences between the two classes of roots arise, it is suggested from differences in the condition of the canes giving rise to each class.

Certain interesting adaptation in sugarcane roots, such as aërotropic curvatures and arrangements for ensuring an efficient rooting, are briefly described.

## CANE-ROOT STUDIES IN THE PHILIPPINES

*Past work.*—The paper of Lee and Bissinger(10) is the only cane-root study that has been published in the Philippines.

## OBJECT OF THE PRESENT WORK

*Purpose explained.*—The root system of cane has been studied principally from its vertical distribution. As a result of these studies, great significance has been attached to the discovery that 70 per cent of the root mass is in the topmost 8 inches of soil.

A complete picture of the root habit of the cane plant cannot be given by distribution studies in one direction only. Therefore, studies in two directions seem desirable.

Also, in spite of Wolters's statement quoted under 1 above, there seems to be some contradictory evidence as to the absolute correlation between shoot and root weights, as affected by fertilizers, and it was thought that parallel determinations of these weights, taken during the early development of the cane plant, both fertilized and unfertilized, would give more conclusive proof of such a dependence.

## METHODS AND MATERIALS USED IN THESE STUDIES

Two parallel series of studies were carried out. One series was with a modified-box method described below, and the other in plots under actual field conditions.

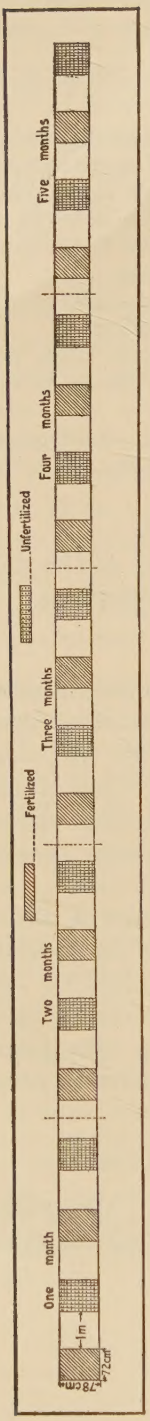


FIG. 1. Showing the arrangement of the modified-box experiment.

*The modified-box method.*—The box method of studying root development of crops has been criticised on the score that conditions obtaining in the boxes are abnormal. In the first place, soil that has been more or less thoroughly aërated is used. In the second place, the soils in the boxes had to be watered to make them compact. The aëration and the watering made the conditions in the boxes more favorable for growth than in the field. However, as it was deemed desirable to actually see the root conformation of the cane, and not simply reconstruct it from the weights found in the different sections both horizontal and vertical, it was decided to carry out the modified-box experiments alongside the field studies. Field conditions were approximated as far as possible. This was accomplished as follows:

*Setting the modified-box experiments.*—On a hillock with a rather abrupt slope, holes 78 and 72 centimeters were dug to a depth of 36 inches (fig. 1). Each 6-inch layer of soil was dug out separately from the other layers. A hillock was selected for the box experiments in order that the washing of the roots at the time of the excavation could be done more easily. Each hole was fitted at the four corners with wooden posts, 8 by 3 by 100 centimeters. To these posts were nailed the five frames holding the wire netting. The holes were filled with soil as follows: First the lowest shelf of wire netting was placed in position and nailed to the posts. Then the corresponding subsoil layer was placed on top of the shelf, packed down, and watered to make the soil set to its original condition. Subsoil was thus put on until the level of the next higher shelf was reached. This second shelf was then put on and similarly nailed to the posts. The corresponding layer of subsoil was again replaced, compacted as in the first layer, and the process was thus continued, layer by layer, until the last shelf and the top-

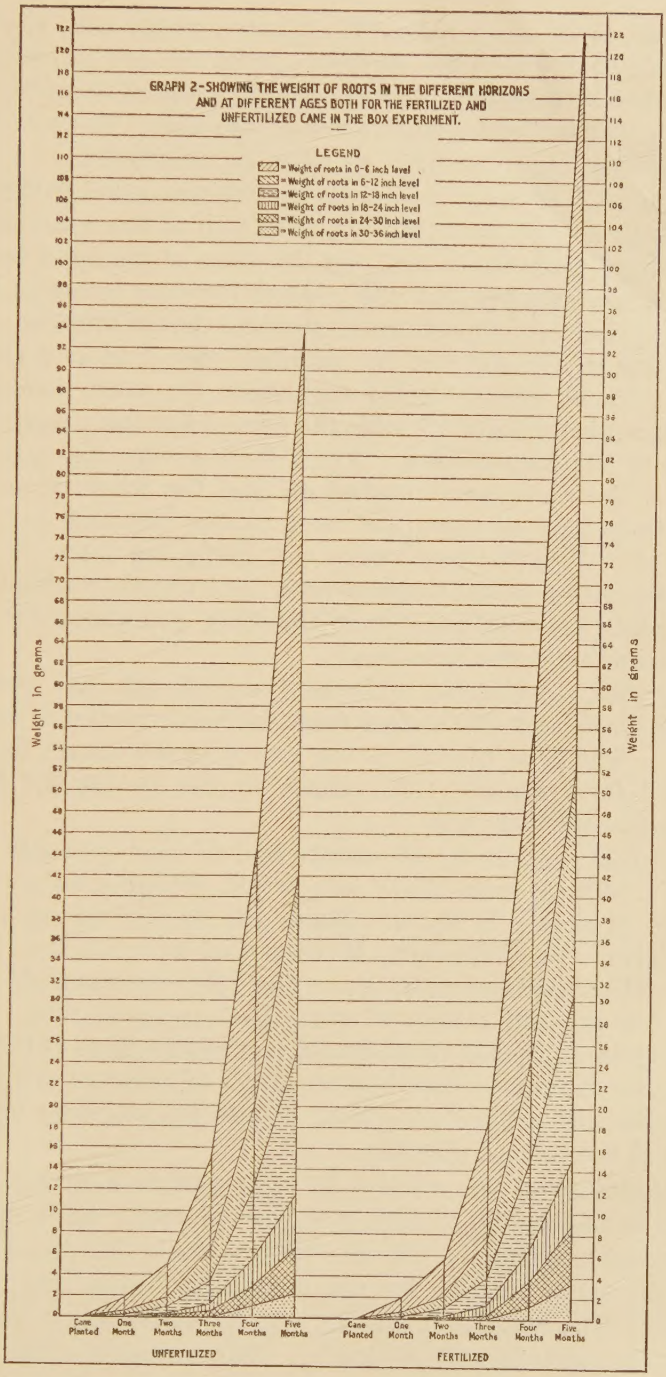
GRAPH 2-SHOWING THE WEIGHT OF ROOTS IN THE DIFFERENT HORIZONS AND AT DIFFERENT AGES BOTH FOR THE FERTILIZED AND UNFERTILIZED CANE IN THE BOX EXPERIMENT.
LEGEND
= Weight of roots in 0-6 inch level
= Weight of roots in 6-12 inch level
= Weight of roots in 12-18 inch level
= Weight of roots in 18-24 inch level
= Weight of roots in 24-30 inch level
= Weight of roots in 30-36 inch level
Weight in grams
Cane Planted
One Month
Two Months
Three Months
Four Months
Five Months
UNFERTILIZED
FERTILIZED

FIG. 2. Showing the weight of roots in the different horizons and at different ages both for the fertilized and unfertilized cane in the box experiment.

most soil layer were replaced. Finally, water was allowed to percolate through all the layers to make the soil as compact as it was before digging. A well-selected point of Mauritius 1900 was planted in the topmost layer of the box. The soil around the growing cane was hoed as often as necessary.

*Excavation of the roots.*—A deep ditch was made at the lowest end of the row of boxes, for the reception of the water and the washed soil from the boxes. As the sides of the holes were left open, it was expected that the roots of the cane extended beyond the wire shelves. Therefore, in starting the excavations, ditches were dug on the sides of the original holes first and any root masses found were collected separately for every 6-inch layer and later placed together with the roots from the corresponding layers in the wire netting. After ditches were dug around the frames, the roots were washed off and the whole frameworks with their load of roots and cane shoots removed and photographed. Then the roots were cut, beginning from the lowest and proceeding to the topmost layer, put together with the corresponding layer collected from the sides, and stored for drying and weighing in the laboratory.

*The field experiment.*—The field experiment was located on level ground some 200 meters from the box experiment described above. The type of soil in the two was the same; light clay derived from volcanic tuff. However, the lot for the field experiment had richer soil.

The field was 50 by 15 meters and divided into lots 5 by 5 meters. There were thirty lots in all, arranged in six groups of five as shown in fig. 5. Three alternating groups received fertilizers. The remaining three served as controls. Twenty-five well-selected points of Mauritius 1900 were planted in each plot, one at each corner of the 1-by-1-meter square. Such a spacing, locally called *dama-dama,* was necessary to reduce to the minimum the competition between roots of adjacent stools. The fertilizer was applied by mixing it with the soil deep below the point at the time of planting.

*Excavation of the roots.*—In each section a group of four stools in the middle of each plot was selected. Each stool was excavated separately. In all, twelve stools for the fertilized and the same number for the unfertilized were dug out each month. The method of digging was by sectioning in two directions, horizontally and vertically. This is shown in fig. 6. The vertical sections were at intervals of 6 inches and are indicated

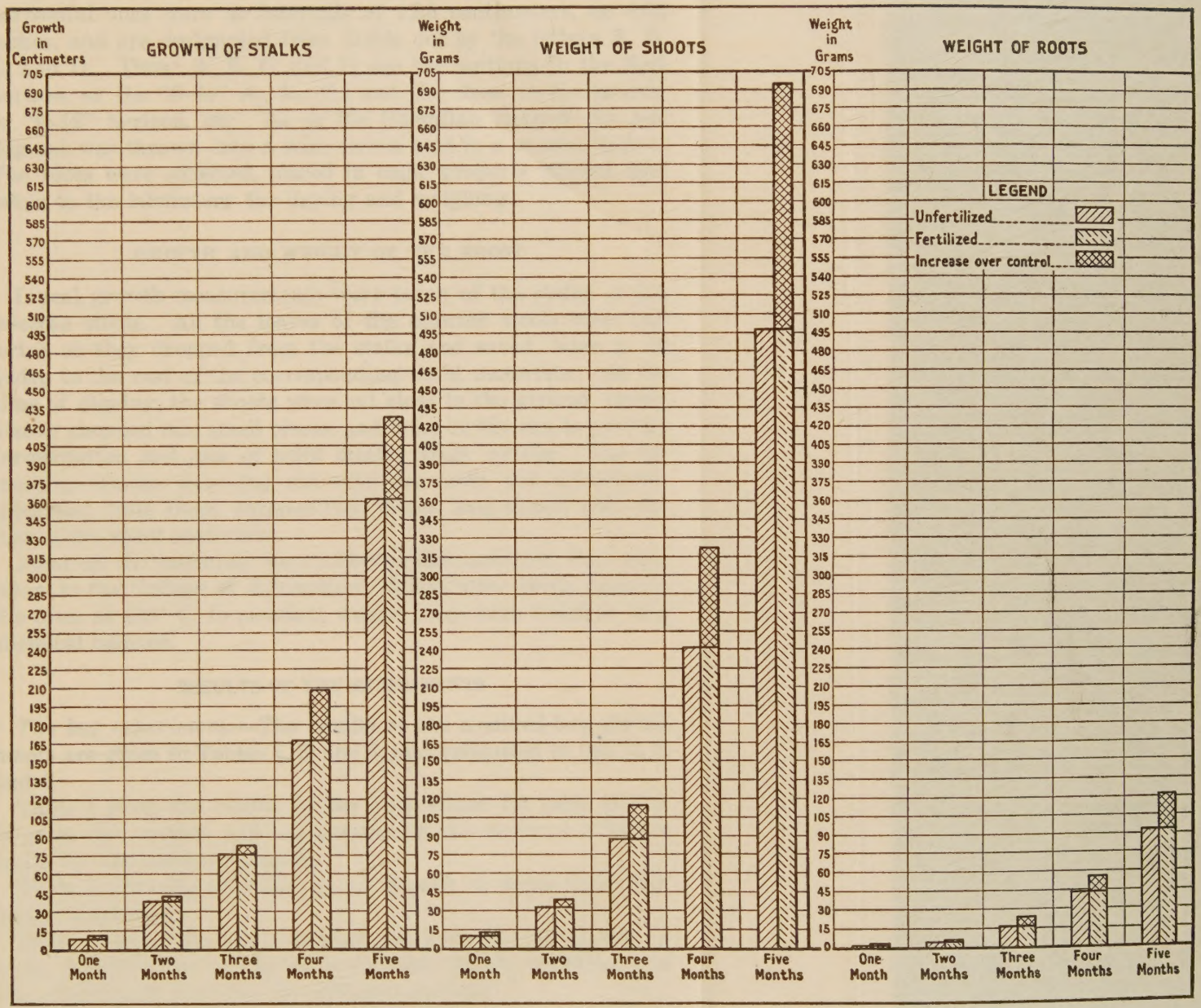


FIG. 3. Comparing growth, weight of shoots, and weight of roots of the fertilized and the unfertilized stools in the modified-box experiment.

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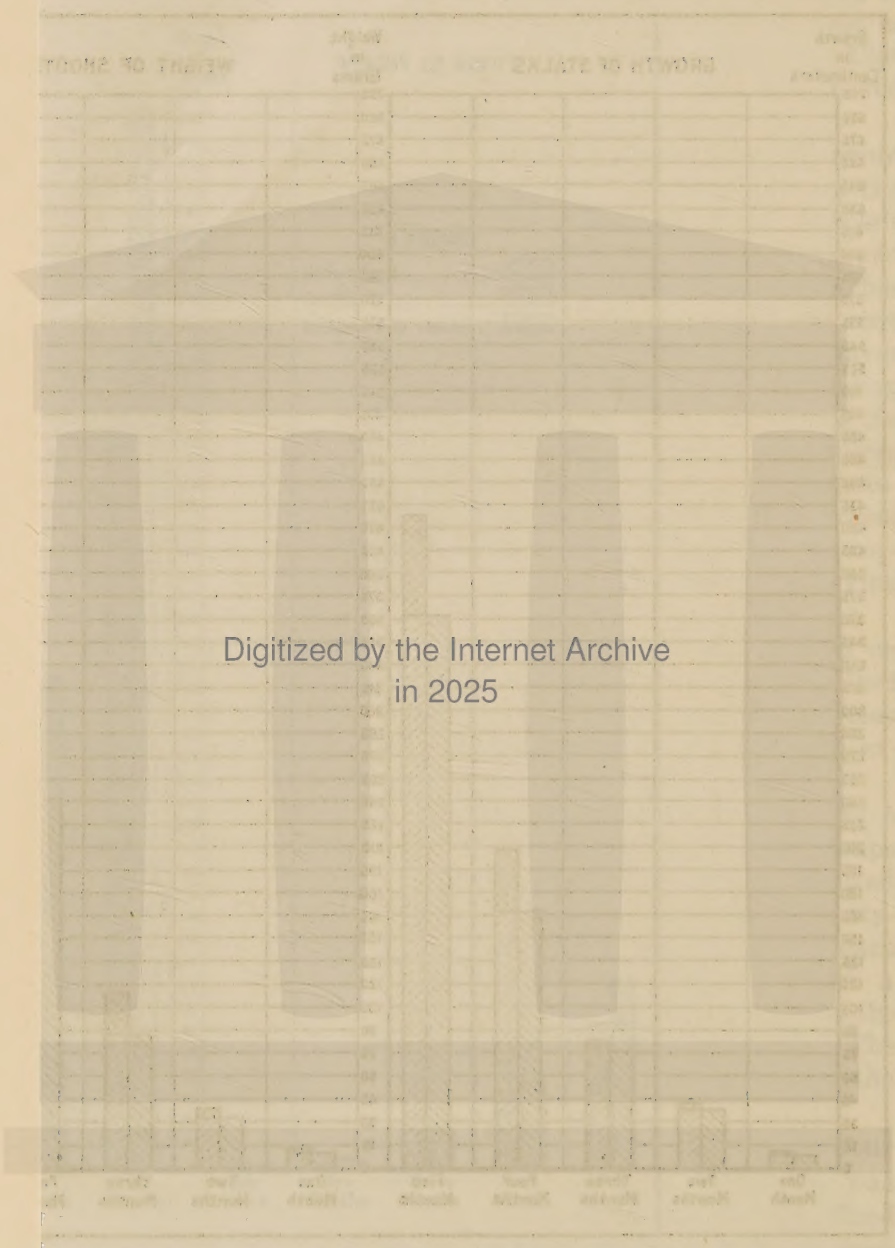

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from top to bottom by subscript numbers 0, 1, 2, 3, etc. The horizontal ones were at intervals of 12.5 centimeters, or 4.92 inches, and are designated from inside out by the letters A, B, C, and D. Thus: A, B, C, and D are the sections in the first horizon, or the "0–6;" $A_1$, $B_1$, $C_1$, and $D_1$; those in the second, or "6–12" horizon, etc. As in the Hawaiian method, the soil dug out was thrown onto a wire screen held in a wooden frame. The roots were collected, placed in bags, properly labeled, and taken to the laboratory for drying and weighing.

## GROWTH AND WEIGHT OF THE SHOOT

Lineal growth measurements were taken of the stalks of the selected stools. All the leaves of the selected stools were collected as they dropped from the stalks and saved, later to be added to the rest of the corresponding shoot materials. At the time of digging, the shoots were cut close to the ground, immediately chopped into small pieces, and dried in the sun to prevent fermentation and loss of solid matter from rotting. The remaining stumps were dug out with the roots, and afterwards separated from them, chipped into pieces, and mixed with the rest of the shoot materials.

After all the materials were collected and sundried, they were taken to the College of Agriculture, where after being dried in the oven at 105° C. to constant weight they were weighed in a chemical balance.

## RESULTS OF THE EXPERIMENTS

*The box experiment.*—The results of the modified-box experiments are given in Tables 1, 2, and 3, and visualized in figs. 2, 3, and 4.

Table 1 gives the results of the excavations for each month. It gives the weights and percentages of the roots at different levels for the different months.

Table 2 gives the average rate of growth of stools under different treatments.

Table 3 gives the total weights per stool of shoots and of roots, and the ratio of tops to roots, both for the fertilized and the unfertilized cane for the different months.

*The field experiments.*—The results of the field experiments are given in Tables 4, 5, and 6. They are visualized in figs. 7, 8, 9, 10, and 11.

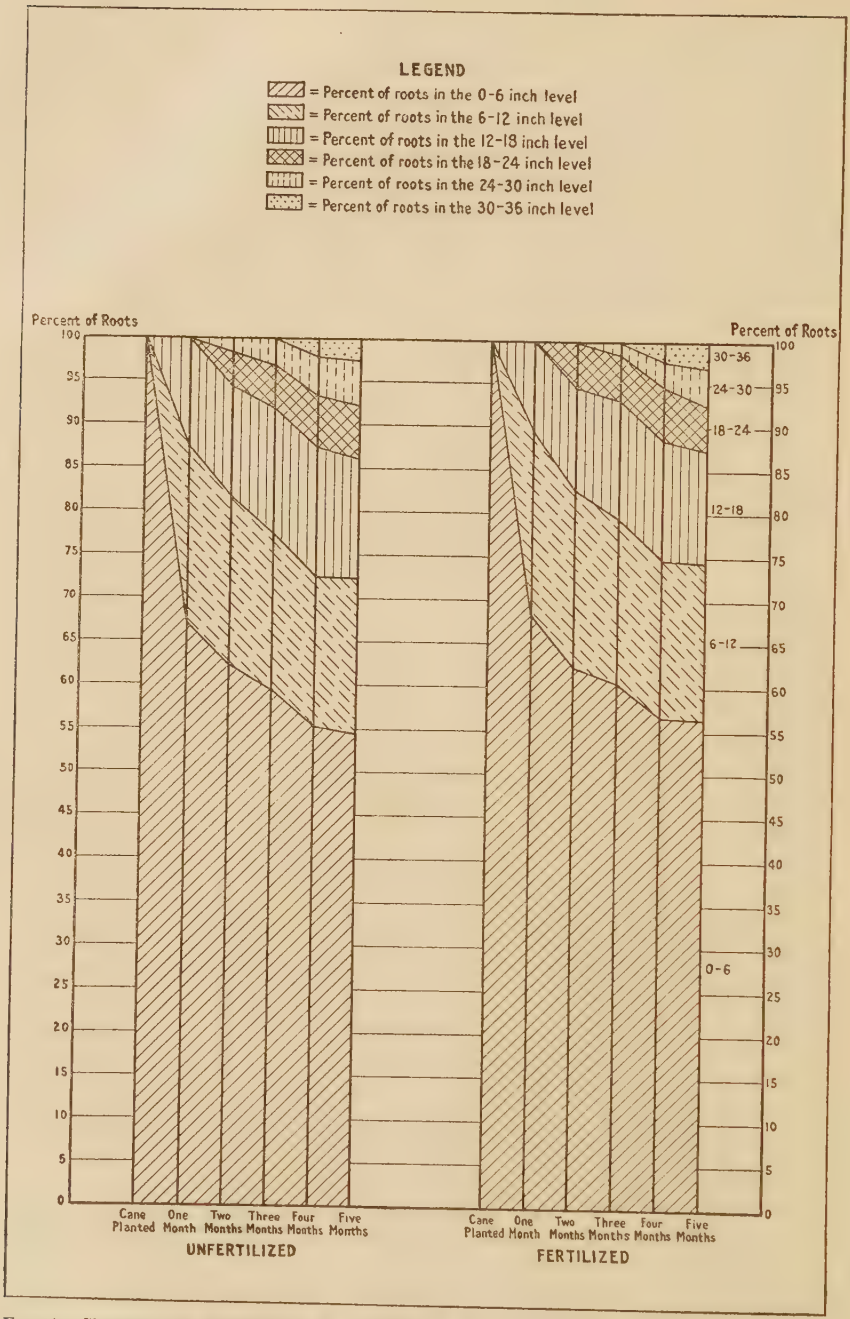


FIG. 4. Showing the distribution in percentages of the total weight of the roots in the different horizons of M-1900 in the modified-box experiment.

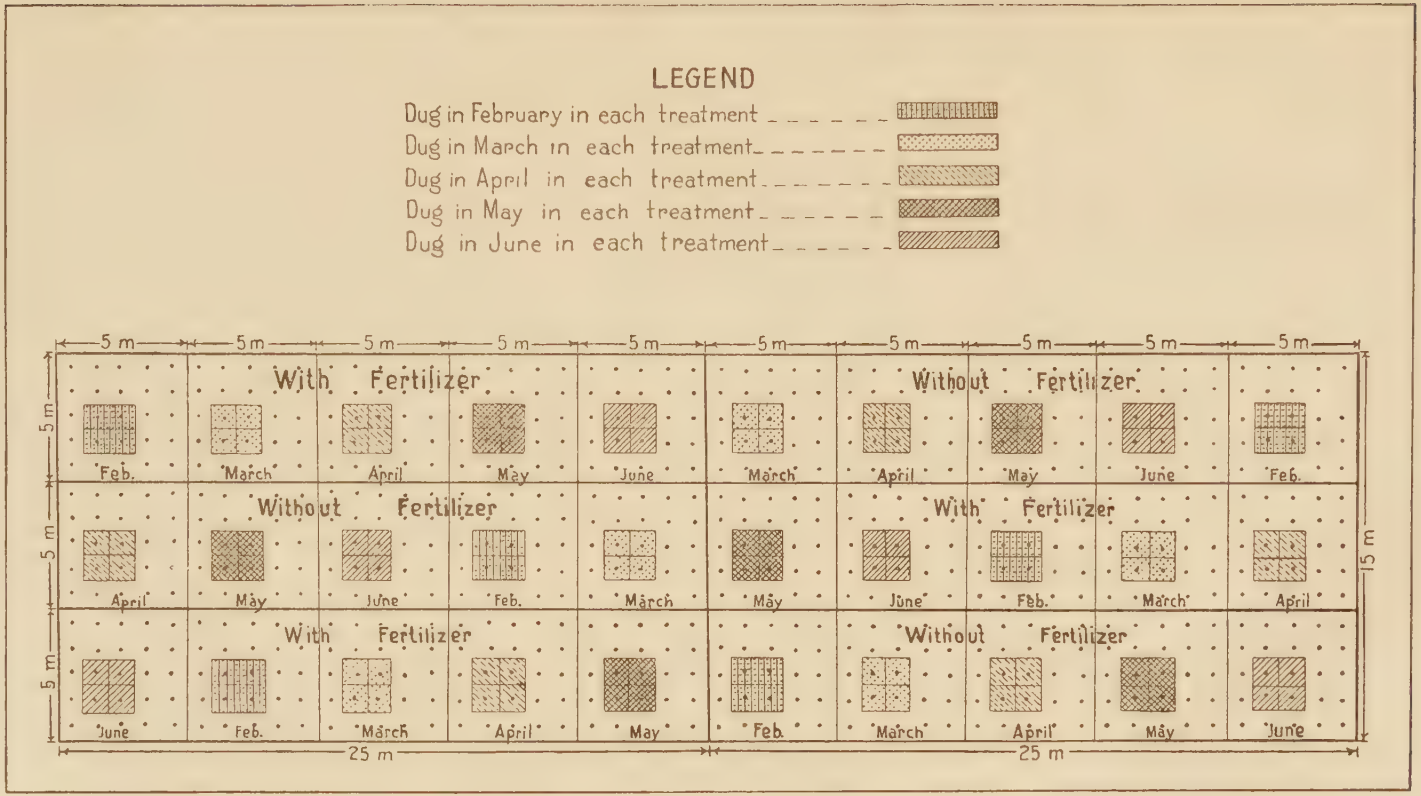


FIG. 5. Showing the arrangement of plots in the experimental field.

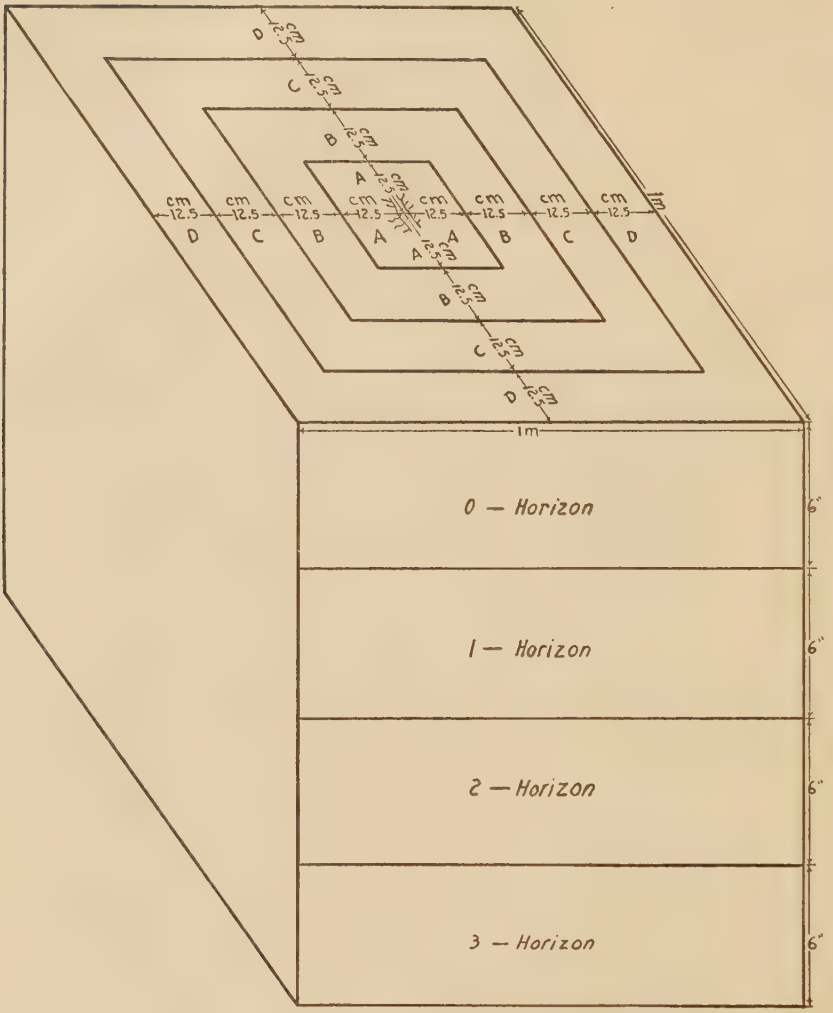


FIG. 6. Showing the different sections of the excavation.

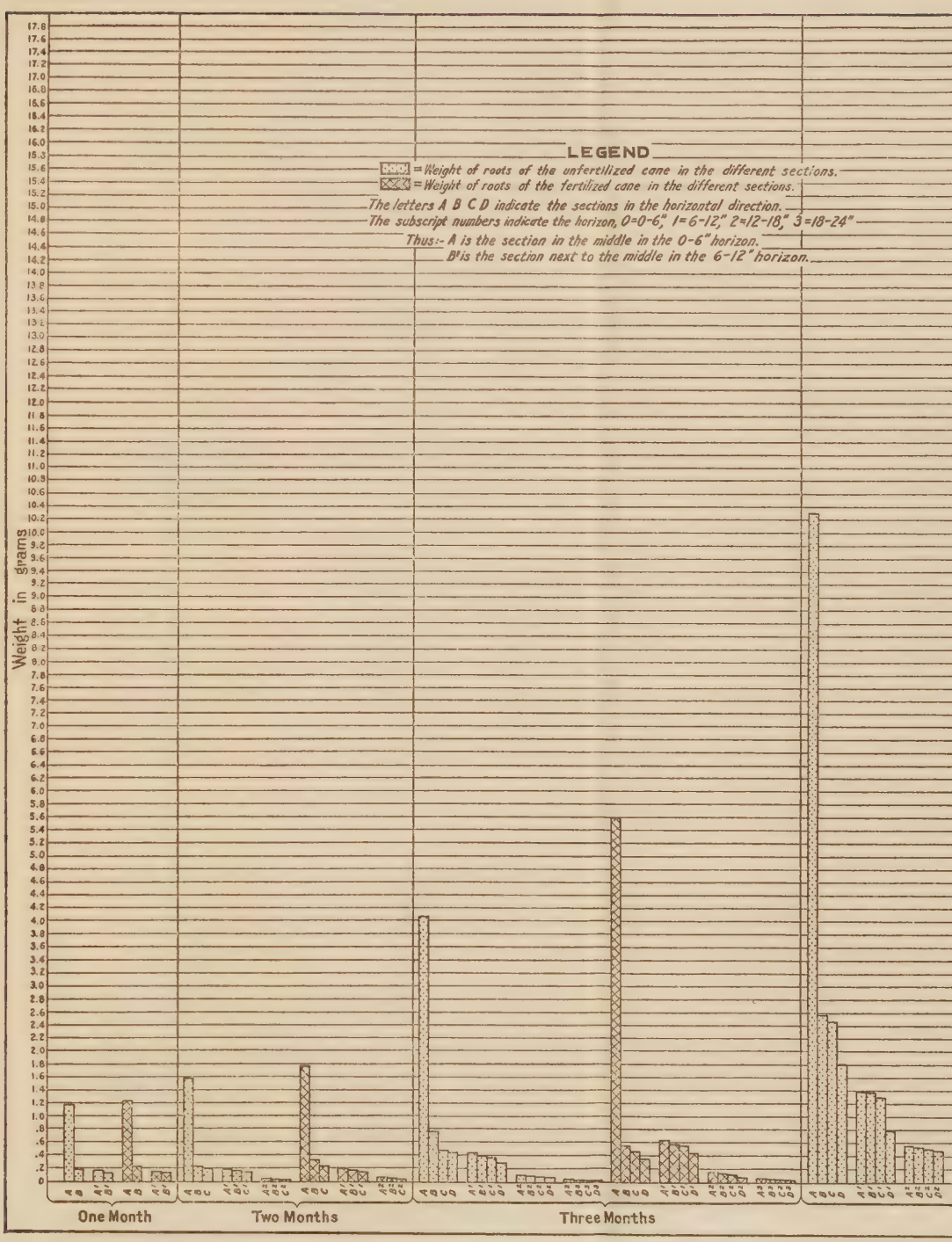
LEGEND
= Weight of roots of the unfertilized cane in the different sections.
= Weight of roots of the fertilized cane in the different sections.
The letters A B C D indicate the sections in the horizontal direction.
The subscript numbers indicate the horizon, 0=0-6", 1=6-12", 2=12-18", 3=18-24"
Thus:- A is the section in the middle in the 0-6" horizon.
B¹ is the section next to the middle in the 6-12" horizon.
Weight in grams
One Month
Two Months
Three Months

FIG. 7. Showing the weight of roots of both the unfertilized and fertilized stools in the differe

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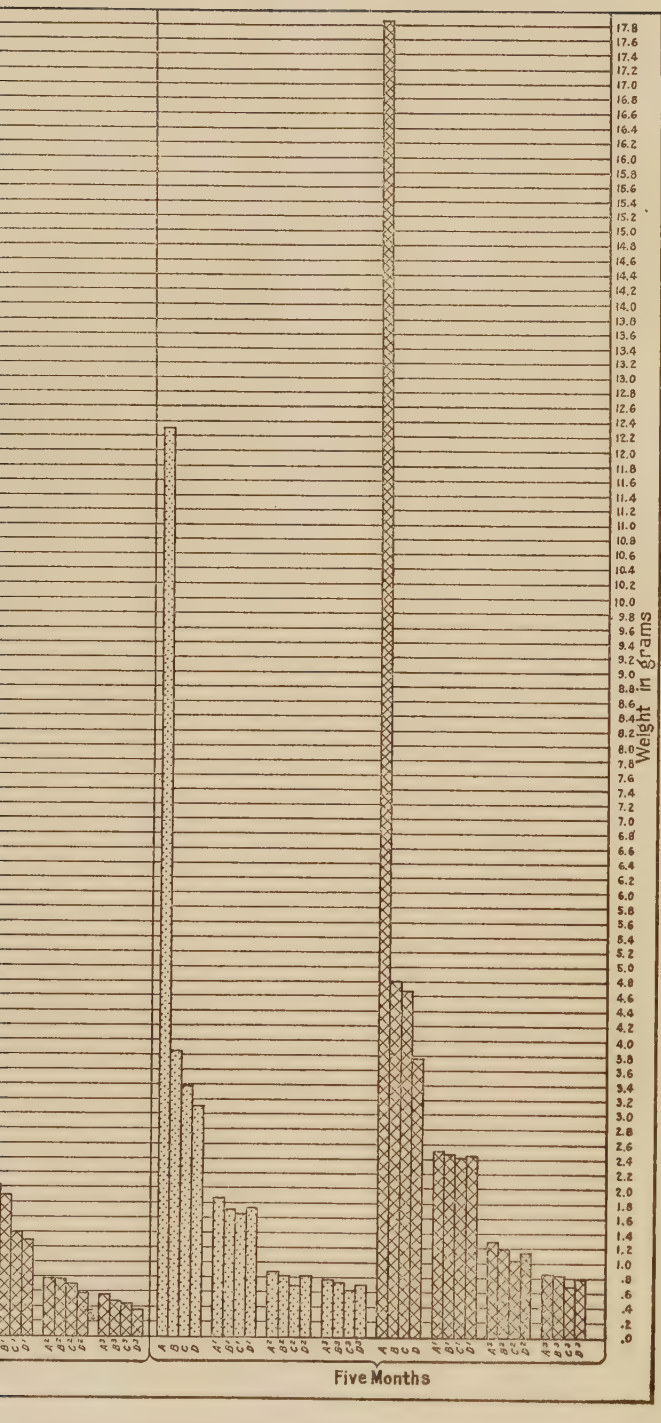


different levels and ages under field conditions.

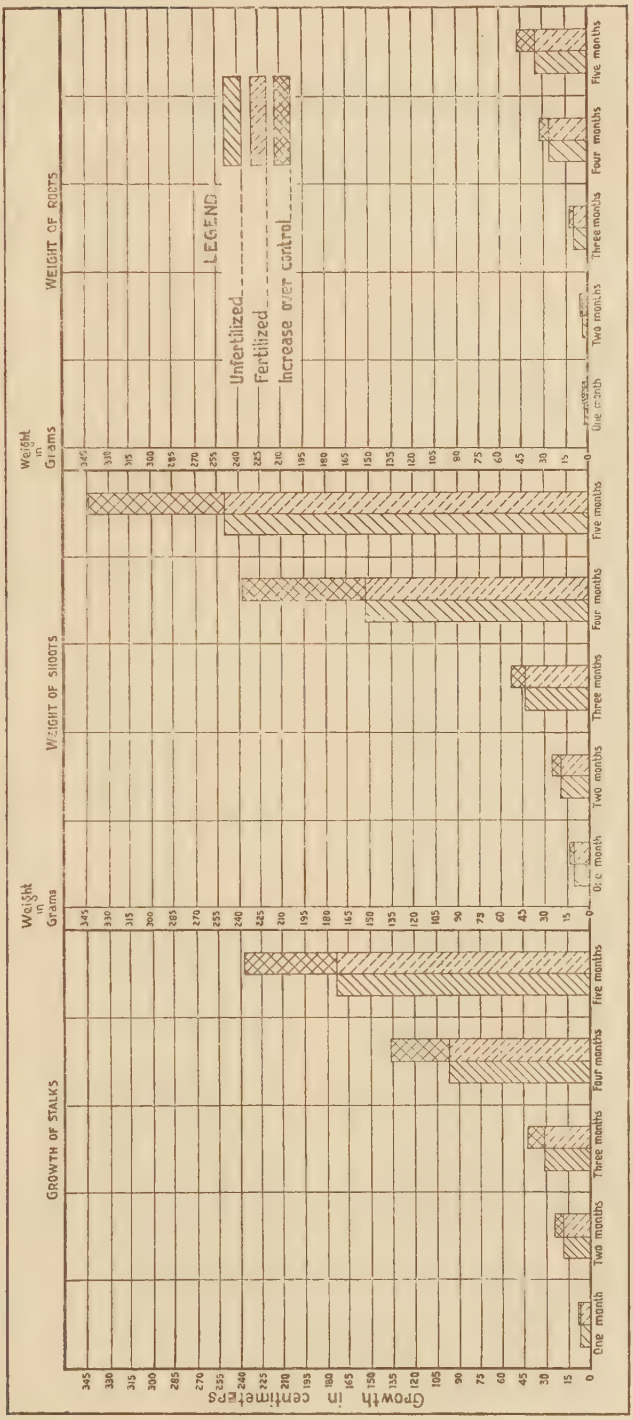


FIG. 8. Comparing the total growth in centimeters, weight of shoots, and weight of roots of both the unfertilized and the fertilized stools at different ages under field conditions.

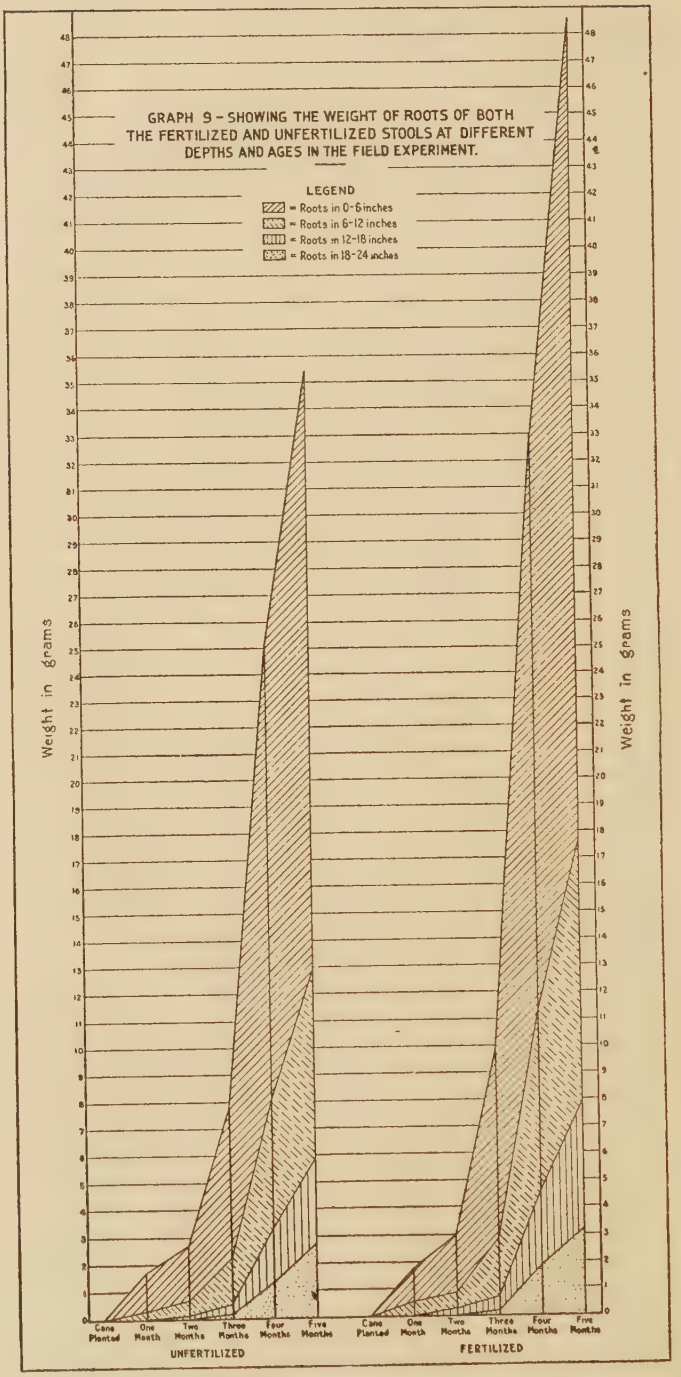
GRAPH 9 – SHOWING THE WEIGHT OF ROOTS OF BOTH THE FERTILIZED AND UNFERTILIZED STOOLS AT DIFFERENT DEPTHS AND AGES IN THE FIELD EXPERIMENT.
LEGEND
= Roots in 0-6 inches
= Roots in 6-12 inches
= Roots in 12-18 inches
= Roots in 18-24 inches
Weight in grams
Weight in grams
Cane Planted
One Month
Two Months
Three Months
Four Months
Five Months
UNFERTILIZED
FERTILIZED

FIG. 9. Showing the weight of roots of both the fertilized and unfertilized stools at different depths and ages in the field experiment.

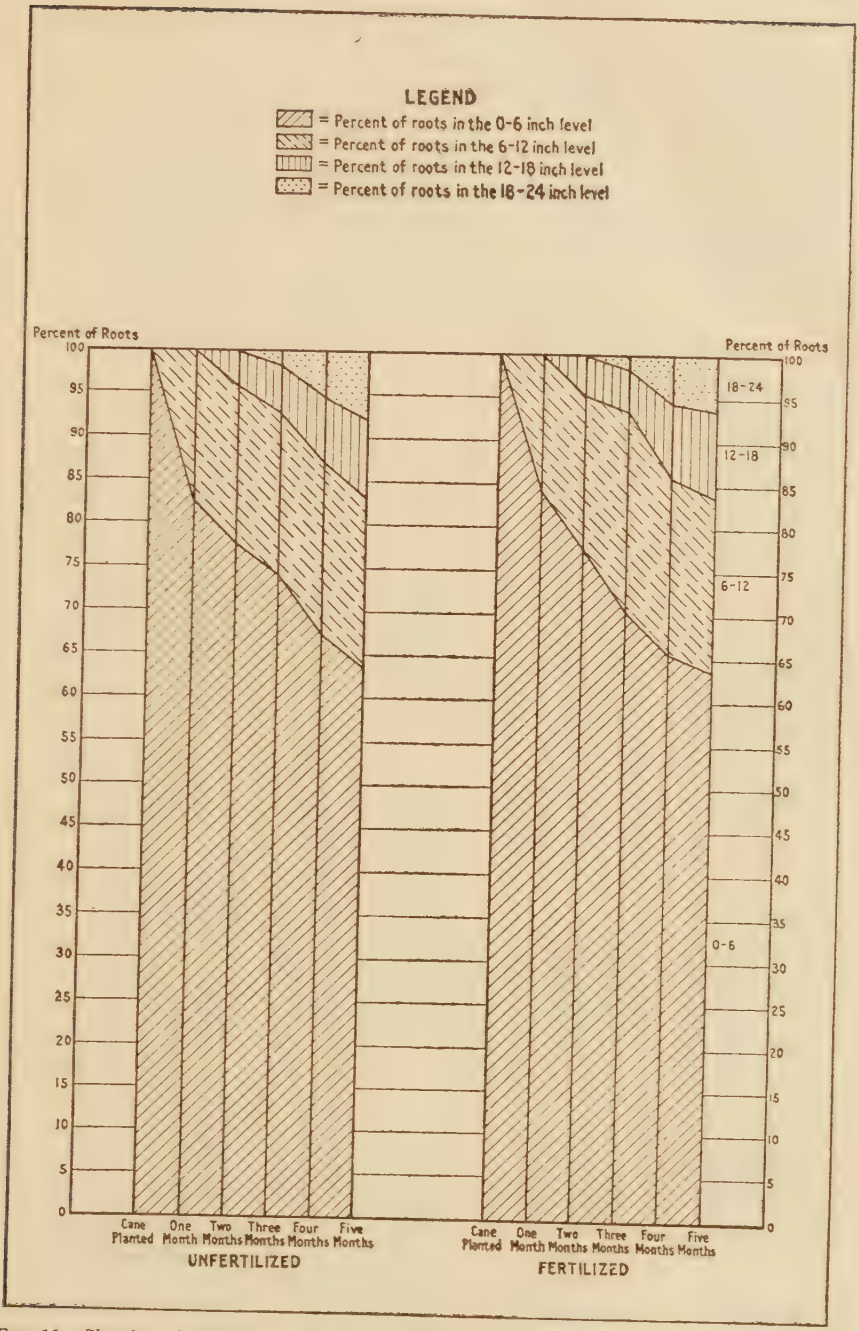


FIG. 10. Showing the distribution in percentages of the total weight of the roots in the different levels of M-1900 under field conditions.

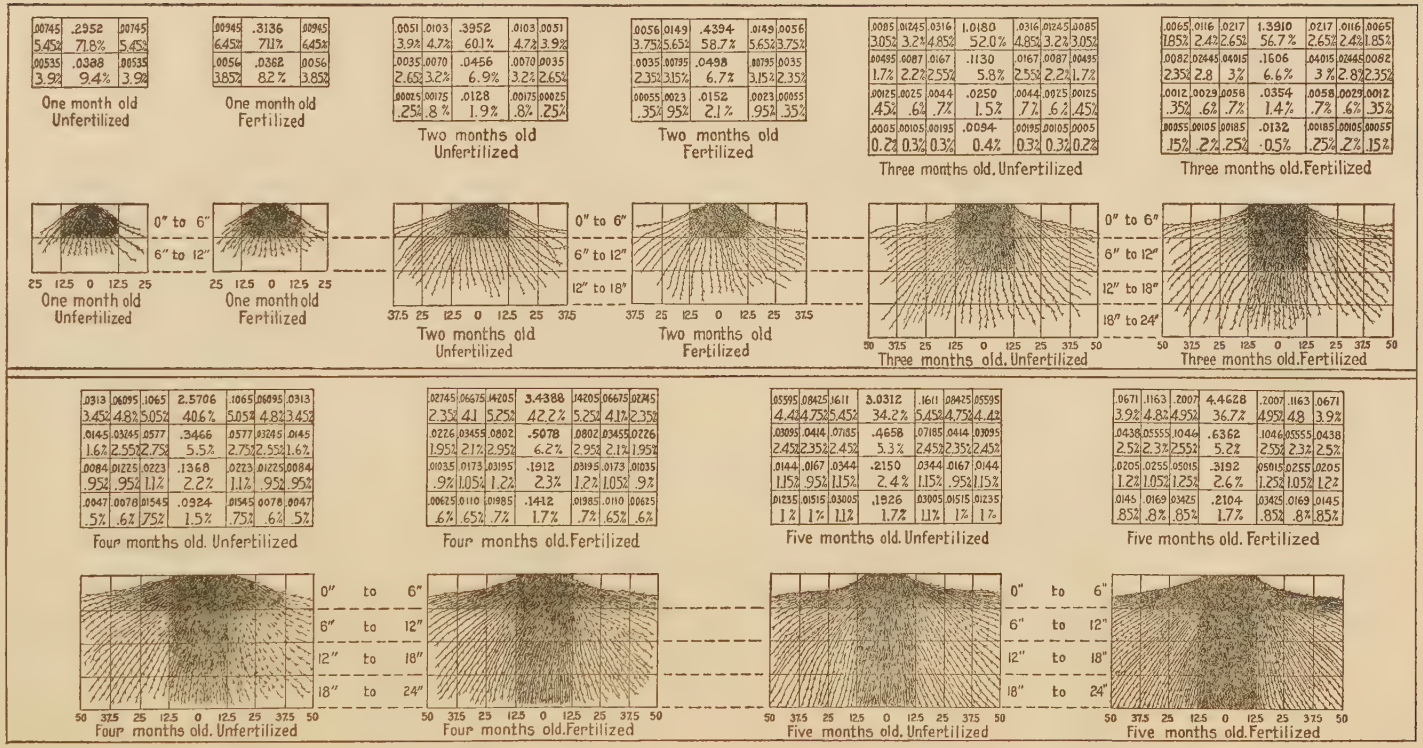


FIG. 11. Showing in figures the weights and percentages of roots in the different sections, and in drawings a section of the root system of M–1900, as reconstructed from its quantitative distribution.

TABLE 1.—*Weights and percentages of roots at different levels and different ages. Modified-box experiment.*

| Age. | Spread of root in wire screen. | Different levels. | Unfertilized. | | Fertilized.[a] | |
|---|---|---|---|---|---|---|
| | | | Weight of roots in the different compartments. | Roots in the different levels in per cent of total weight. | Weight of roots in different compartments. | Roots in different compartments in per cent of total weight. |
| | *cm.* | *in.* | *g.* | | *g.* | |
| One month after planting (January 19 to February 19). | 0–28.5 | 0–6 | 1.2474 | 67.0 | 1.3400 | 68.3 |
| | 0–28.5 | 6–12 | 0.3817 | 20.5 | 0.4080 | 20.8 |
| | 0–28.5 | 6–12 | 0.2308 | 12.4 | 0.2118 | 10.8 |
| Total | | | 1.8599 | 99.9 | 1.9598 | 99.9 |
| Two months after planting (January 19 to March 22). | 0–28 | 0–6 | 3.2309 | 62.8 | 3.5988 | 63.6 |
| | 0–38 | 6–12 | 0.9724 | 18.9 | 1.0807 | 19.1 |
| | 0–38 | 12–18 | 0.6379 | 12.4 | 0.6847 | 12.1 |
| | 0–38 | 18–24 | 0.2056 | 4.0 | 0.2037 | 3.6 |
| | 0–38 | 24–30 | 0.0926 | 1.8 | 0.0848 | 1.5 |
| Total | | | 5.1396 | 99.9 | 5.6527 | 99.9 |
| Three months after planting (January 19 to April 22). | 0–38 | 0–6 | 9.4600 | 59.6 | 11.2762 | 60.3 |
| | 0–38 | 6–12 | 2.8253 | 17.8 | 3.5343 | 18.9 |
| | 0–38 | 12–18 | 2.3015 | 14.5 | 2.5806 | 13.8 |
| | 0–38 | 18–24 | 0.7936 | 5.0 | 0.8976 | 4.8 |
| | 0–38 | 24–30 | 0.4762 | 3.0 | 0.3927 | 2.1 |
| Total | | | 15.8566 | 99.9 | 18.6814 | 99.9 |
| Four months after planting (January 19 to May 22). | 0–47.5 | 0–6 | 24.7018 | 55.3 | 31.9265 | 56.5 |
| | 0–47.5 | 6–12 | 7.5936 | 17.0 | 9.4932 | 16.8 |
| | 0–47.5 | 12–18 | 6.7003 | 15.0 | 8.5325 | 15.1 |
| | 0–47.5 | 18–24 | 2.7247 | 6.1 | 2.9383 | 5.1 |
| | 0–47.5 | 24–30 | 1.8761 | 4.2 | 2.3167 | 4.1 |
| | 0–47.5 | 30–36 | 1.0273 | 2.3 | 1.2432 | 2.2 |
| Total | | | [b] 44.6238 | 99.9 | 56.4504 | 99.9 |
| Five months after planting (January 19 to June 22). | 0–57 | 0–6 | 51.5172 | 54.8 | 69.4535 | 56.8 |
| | 0–57 | 6–12 | 16.8276 | 17.0 | 21.3985 | 17.5 |
| | 0–57 | 12–18 | 12.9732 | 13.8 | 16.0183 | 13.1 |
| | 0–57 | 18–24 | 5.8235 | 6.2 | 6.2361 | 5.1 |
| | 0–57 | 24–30 | 4.5124 | 4.8 | 5.5024 | 4.5 |
| | 0–57 | 30–36 | 2.2562 | 2.4 | 3.5460 | 2.9 |
| Total | | | 93.9151 | 99.9 | 122.1548 | 99.9 |

[a] Weights are average weights from two boxes.

[b] Weight of only one box. The second box was discarded because the cane was attacked by Fiji.

TABLE 2.—*The average growth per week of the unfertilized and fertilized stools at different ages (modified-box experiment).*

[Measurements in centimeters.]

UNFERTILIZED.[a]

| Age. | January 19 planted. | June 26. | February 3 initial measurement. | February 11. | February 19 total growth. | February 26. | March 6. |
|---|---|---|---|---|---|---|---|
| *Months.* | | | | | | | |
| 1 | 0 | 0 | 3.0 | 7.2 | 10.8 | ---- | ---- |
| 2 | 0 | 0 | 3.1 | 7.2 | 11.1 | 18.2 | 22.7 |
| 3 | 0 | 0 | 2.8 | 6.5 | 10.7 | 16.5 | 23.1 |
| 4 | 0 | 0 | 2.0 | 7.4 | 11.8 | 17.4 | 23.9 |
| 5 | 0 | 0 | 1.8 | 6.3 | 11.6 | 19.0 | 28.3 |
| Average [b] | 0 | 0 | 2.5 | 6.9 | 11.2 | 17.8 | 24.5 |
| FERTILIZED.[a] | | | | | | | |
| 1 | 0 | 0 | 3.7 | 6.8 | 12.4 | ---- | ---- |
| 2 | 0 | 0 | 3.7 | 7.1 | 12.3 | 19.4 | 25.3 |
| 3 | 0 | 0 | 3.9 | 7.2 | 11.5 | 18.0 | 25.1 |
| 4 | 0 | 0 | 3.2 | 7.3 | 13.4 | 19.4 | 26.2 |
| 5 | 0 | 0 | 2.1 | 6.1 | 11.4 | 19.6 | 27.6 |
| Average [b] | 0 | 0 | 3.3 | 6.9 | 12.2 | 19.1 | 26.0 |

UNFERTILIZED.[a]

| Age. | March 14. | March 22 total growth. | March 29. | April 6. | April 14. | April 22 total growth. | April 19. |
|---|---|---|---|---|---|---|---|
| *Months.* | | | | | | | |
| 1 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| 2 | 28.6 | 35.3 | ---- | ---- | ---- | ---- | ---- |
| 3 | 30.1 | 38.3 | 44.2 | 50.2 | 56.7 | 62.7 | ---- |
| 4 | 29.0 | 36.5 | 43.5 | 51.5 | 62.5 | 70.6 | 90.6 |
| 5 | 38.3 | 48.0 | 59.6 | 71.0 | 81.5 | 92.9 | 104.7 |
| Average [b] | 31.5 | 39.5 | 49.1 | 57.5 | 66.9 | 75.4 | 97.6 |
| FERTILIZED.[a] | | | | | | | |
| 1 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| 2 | 32.9 | 40.5 | ---- | ---- | ---- | ---- | ---- |
| 3 | 32.0 | 39.5 | 46.8 | 53.5 | 60.4 | 68.5 | ---- |
| 4 | 31.5 | 40.6 | 48.2 | 57.7 | 66.6 | 78.4 | 102.5 |
| 5 | 37.0 | 49.4 | 61.4 | 77.5 | 90.1 | 107.2 | 128.8 |
| Average [b] | 33.3 | 43.5 | 52.1 | 62.9 | 72.3 | 84.7 | 115.6 |

[a] In every month, the average of twelve measurements was taken.

[b] First month, one measurement; second month, average of eight measurements; third month, average of six measurements; fourth month, average of four measurements; fifth month, average of two measurements.

TABLE 2.—*The average growth per week of the unfertilized and fertilized stools at different ages (modified-box experiment)*—Continued.

UNFERTILIZED.[a]

| Age. | May 6. | May 14. | May 22 total growth. | May 30. | June 6. | June 14. | June 22 total growth. |
|---|---|---|---|---|---|---|---|
| *Months.* | | | | | | | |
| 1 | | | | | | | |
| 2 | | | | | | | |
| 3 | | | | | | | |
| 4 | 111.8 | 126.0 | 161.0 | | | | |
| 5 | 126.3 | 145.3 | 176.4 | 200.6 | 259.7 | 303.7 | 364.1 |
| Average [b] | 119.0 | 135.6 | 168.7 | 200.6 | 259.7 | 303.7 | 364.1 |
| FERTILIZED.[a] | | | | | | | |
| 1 | | | | | | | |
| 2 | | | | | | | |
| 3 | | | | | | | |
| 4 | 122.8 | 165.7 | 195.2 | | | | |
| 5 | 152.9 | 195.5 | 223.5 | 263.4 | 314.0 | 370.9 | 428.5 |
| Average [b] | 137.8 | 180.6 | 209.4 | 263.4 | 314.0 | 370.9 | 428.5 |

[a] In every month, the average of twelve measurements was taken.

[b] First month, one measurement; second month, average of eight measurements; third month, average of six measurements; fourth month, average of four measurements; fifth month, average of two measurements.

TABLE 3.—*Weights per stool of shoots; ratio of tops to roots in the modified-box experiment.*[a]

| Treatment. | Average growth of shoots per stool each month. | Average weight of shoots per stool each month. | Average weight of roots per stool each month. | Ratio of tops to roots. |
|---|---|---|---|---|
| ONE MONTH | *cm.* | *g.* | *g.* | |
| Fertilized | 12.2 | 14.6985 | 1.9598 | 7.5 |
| Unfertilized | 11.2 | 13.3526 | 1.8599 | 7.2 |
| Increase over control | 1.0 | 1.3459 | 0.0999 | ---------- |
| Do.....per cent.. | 8.9 | 10.7 | 5.3 | ---------- |
| TWO MONTHS | | | | |
| Fertilized | 43.5 | 39.0036 | 5.6527 | 6.9 |
| Unfertilized | 39.5 | 33.4074 | 5.1396 | 6.5 |
| Increase over control | 4.0 | 5.5962 | 0.5131 | ---------- |
| Do.....per cent.. | 10.1 | 16.7 | 9.9 | ---------- |
| THREE MONTHS | | | | |
| Fertilized | 84.7 | 115.8246 | 18.6814 | 6.2 |
| Unfertilized | 75.4 | 88.7969 | 15.8566 | 5.6 |
| Increase over control | 9.3 | 27.0277 | 2.8248 | ---------- |
| Do.....per cent.. | 12.3 | 30.4 | 17.8 | ---------- |
| FOUR MONTHS | | | | |
| Fertilized | 209.4 | 327.4123 | 56.4504 | 5.8 |
| Unfertilized | 168.7 | 240.9685 | 44.6238 | 5.4 |
| Increase over control | 40.4 | 86.4438 | 11.8266 | ---------- |
| Do.....per cent.. | 23.9 | 35.8 | 26.5 | ---------- |
| FIVE MONTHS | | | | |
| Fertilized | 428.5 | 696.2823 | 122.1548 | 5.7 |
| Unfertilized | 364.2 | 497.7500 | 93.9151 | 5.3 |
| Increase over control | 64.3 | 198.5323 | 28.2397 | ---------- |
| Do.....per cent.. | 17.6 | 39.8 | 30.0 | ---------- |

[a] Fifteen grams per point.

TABLE 4.—*Weights and percentages of roots of unfertilized and fertilized stools at different ages and different sections in the field experiments.*

| Age. | Section. | Lateral spread. | Depth. | Unfertilized. | | | | Fertilized. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Average weight of roots.[a] | Total weight of roots at different depths. | Roots in each section per cent total roots. | Roots at different depths per cent total roots. | Average weight of roots.[a] | Total weight of roots at different depths. | Roots in each section per cent total roots. | Roots at different depths per cent total roots. |
| | | *cm.* | *in.* | *g.* | | | | *g.* | | | |
| One month after planting | $A_1$ | 0–12.5 | 0–6 | 1.1813 | | 71.8 | | 1.2550 | | 71.1 | |
| | B | 12.5–25 | 0–6 | 0.1796 | 1.3609 | 10.9 | 82.7 | 0.2276 | 1.4826 | 12.9 | 84.0 |
| | $A_1$ | 0–12.5 | 6–12 | 0.1555 | | 9.4 | | 0.1452 | | 8.2 | |
| | $B_1$ | 12.5–25 | 6–12 | 0.1266 | 0.2841 | 7.8 | 17.2 | 0.1355 | 0.2807 | 7.7 | 15.9 |
| Total | | | | 1.6450 | 1.6450 | 99.9 | 99.9 | 1.7633 | 1.7633 | 99.9 | 99.9 |
| Two months after planting | A | 0–12.5 | 0–6 | 1.5811 | | 60.1 | | 1.7580 | | 58.7 | |
| | B | 12.5–25 | 0–6 | 0.2477 | | 9.4 | | 0.3383 | | 11.3 | |
| | C | 25–37.5 | 0–6 | 0.2047 | 2.0335 | 7.8 | 77.3 | 0.2255 | 2.3218 | 7.5 | 77.6 |
| | $A_1$ | 0–12.5 | 6–12 | 0.1830 | | 6.9 | | 0.1995 | | 6.7 | |
| | $B_1$ | 12.5–5–25 | 6–12 | 0.1680 | | 6.4 | | 0.1913 | | 6.3 | |
| | $C_1$ | 25–37.5 | 6–12 | 0.1404 | 0.4914 | 5.3 | 18.6 | 0.1400 | 0.5308 | 4.7 | 17.7 |
| | $A_2$ | 0–12.5 | 12–18 | 0.0513 | | 1.9 | | 0.0615 | | 2.1 | |
| | $B_2$ | 12.5–25 | 12–18 | 0.0428 | | 1.6 | | 0.0556 | | 1.9 | |
| | $C_2$ | 25–37.5 | 12–18 | 0.0116 | 0.1057 | 0.5 | 4.0 | 0.0236 | 0.1407 | 0.7 | 4.6 |
| Total | | | | 2.6306 | 2.6306 | 99.9 | 99.9 | 2.9933 | 2.9933 | 99.9 | 99.9 |
| Three months after planting | A | 0–12.5 | 0–6 | 4.0721 | | 52.0 | | 5.5647 | | 56.7 | |
| | B | 12.5–25 | 0–6 | 0.7592 | | 9.7 | | 0.5209 | | 5.3 | |
| | C | 25–37.5 | 0–6 | 0.4984 | | 6.4 | | 0.4713 | | 4.8 | |
| | D | 37.5–50 | 0–6 | 0.4773 | 5.8050 | 6.1 | 74.2 | 0.3655 | 6.9224 | 3.7 | 70.6 |
| | $A_1$ | 0–12.5 | 6–12 | 0.4526 | | 5.8 | | 0.6428 | | 6.6 | |
| | $B_1$ | 12.5–25 | 6–12 | 0.4014 | | 5.1 | | 0.5879 | | 6.0 | |

[a] Twelve stools were averaged.

TABLE 4.—*Weights and percentages of stools of unfertilized and fertilized stools at different ages and different sections in the field experiments*—Continued.

| Age | Section. | Lateral spread. | Depth. | Unfertilized. | | | | Fertilized. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Average weight of roots. [a] | Total weight of roots at different depths. | Roots in each section per cent total roots. | Roots at different depths per cent total roots. | Average weight of roots. [a] | Total weight of roots at different depths. | Roots in each section per cent total roots. | Roots at different depths per cent total roots. |
| | | *cm.* | *in.* | *g.* | | | | *g.* | | | |
| Three months after planting | $C_1$ | 25–37.5 | 6–12 | 0.3481 | | 4.4 | | 0.5475 | | 5.6 | |
| | $D_1$ | 37.5–50 | 6–12 | 0.2774 | 1.4795 | 3.4 | 18.7 | 0.4599 | 2.2382 | 4.7 | 22.8 |
| | $A_2$ | 0–12.5 | 12–18 | 0.1003 | | 1.5 | | 0.1418 | | 1.4 | |
| | $B_2$ | 12.5–25 | 12–18 | 0.1064 | | 1.4 | | 0.1398 | | 1.4 | |
| | $C_2$ | 25–37.5 | 12–18 | 0.1003 | | 1.2 | | 0.1162 | | 1.2 | |
| | $D_2$ | 37.5–50 | 12–18 | 0.0707 | 0.3777 | 0.9 | 5.1 | 0.0679 | 0.4657 | 0.7 | 4.8 |
| | $A_3$ | 0–12.5 | 18–24 | 0.0377 | | 0.4 | | 0.0531 | | 0.5 | |
| | $B_3$ | 12.5–25 | 18–24 | 0.0475 | | 0.6 | | 0.0453 | | 0.3 | |
| | $C_3$ | 25–37.5 | 18–24 | 0.0433 | | 0.6 | | 0.0433 | | 0.4 | |
| | $D_3$ | 37.5–50 | 18–24 | 0.0291 | 0.1576 | 0.4 | 1.9 | 0.0550 | 0.1747 | 0.3 | 1.7 |
| Total | | | | 7.8198 | 7.8198 | 99.9 | 99.9 | 9.8010 | 9.8010 | 99.9 | 99.9 |
| Four months after planting | A | 0–12.5 | 0–6 | 10.2828 | | 40.6 | | 13.7557 | | 42.2 | |
| | B | 12.5–25 | 0–6 | 2.5565 | | 10.1 | | 3.4102 | | 10.5 | |
| | C | 25–37.5 | 0–6 | 2.4394 | | 9.6 | | 2.6716 | | 8.2 | |
| | D | 37.5–50 | 0–6 | 1.7531 | 17.0318 | 6.9 | 67.3 | 1.5376 | 21.3751 | 4.7 | 65.5 |
| | $A_1$ | 0–12.5 | 6–12 | 1.3871 | | 5.5 | | 2.0317 | | 6.2 | |
| | $B_1$ | 12.5–25 | 6–12 | 1.3856 | | 5.5 | | 1.9259 | | 5.9 | |
| | $C_1$ | 25–37.5 | 6–12 | 1.2996 | | 5.1 | | 1.3839 | | 4.2 | |
| | $D_1$ | 37.5–50 | 6–12 | 0.8120 | 4.8845 | 3.2 | 19.3 | 1.2668 | 6.6083 | 3.9 | 20.3 |
| | $A_2$ | 0–12.5 | 12–18 | 0.5479 | | 2.2 | | 0.7652 | | 2.3 | |
| | $B_2$ | 12.5–25 | 12–18 | 0.5356 | | 2.2 | | 0.7669 | | 2.4 | |
| | $C_2$ | 25–37.5 | 12–18 | 0.4917 | | 1.9 | | 0.6926 | | 2.1 | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Four months after planting | $D_2$ | 37.5–50 | 12–18 | 0.4729 | 2.0481 | 1.9 | 8.1 | 0.5806 | 2.8053 | 1.8 | 8.6 |
| | $A_3$ | 0–12.5 | 18–24 | 0.3698 | | 1.5 | | 0.5654 | | 1.7 | |
| | $B_3$ | 12.5–25 | 18–24 | 0.3718 | | 1.5 | | 0.4769 | | 1.4 | |
| | $C_3$ | 25–37.5 | 18–24 | 0.3129 | | 1.2 | | 0.4408 | | 1.3 | |
| | $D_3$ | 37.5–50 | 18–24 | 0.2641 | 1.3186 | 1.0 | 5.2 | 0.3517 | 1.8548 | 1.2 | 5.6 |
| Total | | | | 25.2828 | 26.2828 | 99.9 | 99.9 | 32.6235 | 32.6235 | 100.0 | 100.0 |
| Five months after planting | A | 0–12.5 | 0–6 | 12.1254 | | 34.2 | | 17.8516 | | 36.7 | |
| | B | 12.5–25 | 0–6 | 3.8668 | | 10.9 | | 4.8170 | | 9.9 | |
| | C | 25–37.5 | 0–6 | 3.3713 | | 9.5 | | 4.6535 | | 9.6 | |
| | D | 37.5–50 | 0–6 | 3.1334 | 22.4969 | 8.8 | 63.5 | 3.7593 | 31.0814 | 7.8 | 63.9 |
| | $A_1$ | 0–12.5 | 6–12 | 1.8632 | | 5.3 | | 2.5455 | | 5.2 | |
| | $B_1$ | 12.5–25 | 6–12 | 1.7244 | | 4.9 | | 2.5106 | | 5.1 | |
| | $C_1$ | 25–37.5 | 6–12 | 1.6569 | | 4.7 | | 2.2226 | | 4.6 | |
| | $D_1$ | 37.5–50 | 6–12 | 1.7345 | 6.9790 | 4.9 | 19.7 | 2.4534 | 9.7321 | 5.0 | 20.0 |
| | $A_2$ | 0–12.5 | 12–18 | 0.8604 | | 2.4 | | 1.2772 | | 2.6 | |
| | $B_2$ | 12.5–25 | 12–18 | 0.8260 | | 2.3 | | 1.2036 | | 2.5 | |
| | $C_2$ | 25–37.5 | 12–18 | 0.6642 | | 1.9 | | 1.0289 | | 2.1 | |
| | $D_2$ | 37.5–50 | 12–18 | 0.8071 | 3.1577 | 2.3 | 8.9 | 1.1481 | 4.6578 | 2.4 | 9.6 |
| | $A_3$ | 0–12.5 | 18–24 | 0.7707 | | 1.7 | | 0.8417 | | 1.7 | |
| | $B_3$ | 12.5–25 | 18–24 | 0.7217 | | 2.2 | | 0.8227 | | 1.7 | |
| | $C_3$ | 25–37.5 | 18–24 | 0.6070 | | 2.0 | | 0.6772 | | 1.6 | |
| | $D_3$ | 37.5–50 | 18–24 | 0.6933 | 2.7927 | 2.0 | 7.9 | 0.8121 | 3.1537 | 1.7 | 6.5 |
| Total | | | | 35.4263 | 35.4263 | 100.0 | 100.0 | 48.6250 | 48.6250 | 99.9 | 100.0 |

[a] Twelve stools were averaged.

TABLE 4*a*.—*Densities in grams of roots per block for different ages, both for the unfertilized and fertilized stools. Densities (grams per hectare).*[a]

| Age. | Block. | Density. | |
|---|---|---|---|
| | | Unfertilized. | Fertilized. |
| One month after planting | A | 0.2943 | 0.3136 |
| | B | 0.01496 | 0.0189 |
| | $A_1$ | 0.0398 | 0.0362 |
| | $B_1$ | 0.01071 | 0.0112 |
| Two months after planting | A | 0.3952 | 0.4394 |
| | B | 0.02065 | 0.0298 |
| | C | 0.01024 | 0.0112 |
| | $A_1$ | 0.0457 | 0.0498 |
| | $B_1$ | 0.0140 | 0.0159 |
| | $C_1$ | 0.00702 | 0.0070 |
| | $A_2$ | 0.0129 | 0.0152 |
| | $B_2$ | 0.0035 | 0.0046 |
| | $C_2$ | 0.0005 | 0.0011 |
| Three months after planting | A | 1.0180 | 1.3910 |
| | B | 0.0632 | 0.0434 |
| | C | 0.0240 | 0.0232 |
| | D | 0.0170 | 0.0130 |
| | $A_1$ | 0.1130 | 0.1606 |
| | $B_1$ | 0.0334 | 0.0489 |
| | $C_1$ | 0.0174 | 0.0273 |
| | $D_1$ | 0.0099 | 0.0164 |
| | $A_2$ | 0.0250 | 0.0354 |
| | $B_2$ | 0.0088 | 0.0116 |
| | $C_2$ | 0.0050 | 0.0058 |
| | $D_2$ | 0.0025 | 0.0024 |
| | $A_3$ | 0.0094 | 0.0132 |
| | $B_3$ | 0.0039 | 0.0037 |
| | $C_3$ | 0.0021 | 0.0021 |
| | $D_3$ | 0.0010 | 0.0011 |
| Four months after planting | A | 2.5700 | 3.4398 |
| | B | 0.2130 | 0.2841 |
| | C | 0.1219 | 0.1336 |
| | D | 0.0626 | 0.0549 |
| | $A_1$ | 0.3467 | 0.5078 |
| | $B_1$ | 0.11546 | 0.1604 |
| | $C_1$ | 0.06498 | 0.0691 |
| | $D_1$ | 0.0290 | 0.0452 |
| | $A_2$ | 0.1369 | 0.1913 |
| | $B_2$ | 0.04463 | 0.0639 |
| | $C_2$ | 0.0245 | 0.0346 |
| | $D_2$ | 0.0168 | 0.0207 |
| | $A_3$ | 0.0924 | 0.1413 |
| | $B_3$ | 0.0309 | 0.0397 |
| | $C_3$ | 0.0156 | 0.0220 |
| | $D_3$ | 0.0094 | 0.0125 |

[a] The densities given in this table are calculated as follows: The weights of roots for all the A-sections were divided by 4, those for the B's by 12, those for the C's by 20, and those for the D's by 28. Those are the numbers of blocks of 12.5 centimeters and 6 inches contained in those sections in the different horizons.

TABLE 4*a*.—*Densities in grams of roots per block for different ages, both for the unfertilized and fertilized stools. Densities (grams per hectare)*—Continued.

| Age. | Block. | Density. | |
|---|---|---|---|
| | | Unfertilized. | Fertilized. |
| Five months after planting | A | 3.0312 | 4.4629 |
| | B | 0.3222 | 0.4014 |
| | C | 0.1685 | 0.2326 |
| | D | 0.1119 | 0.1342 |
| | $A_1$ | 0.4658 | 0.6362 |
| | $B_1$ | 0.1437 | 0.2092 |
| | $C_1$ | 0.0828 | 0.1111 |
| | $D_1$ | 0.0619 | 0.0876 |
| | $A_2$ | 0.2050 | 0.3193 |
| | $B_2$ | 0.0688 | 0.1003 |
| | $C_2$ | 0.0334 | 0.0514 |
| | $D_2$ | 0.0288 | 0.0410 |
| | $A_3$ | 0.1926 | 0.0104 |
| | $B_3$ | 0.0601 | 0.0685 |
| | $C_3$ | 0.0603 | 0.0338 |
| | $D_3$ | 0.0247 | 0.0290 |

TABLE 5.—*Average growth per week of the unfertilized and fertilized stools at different ages in the field experiments.*

UNFERTILIZED.[a]

| Age. | January 19 planted. | January 26. | February 3 initial measurements. | February 11. | February 19 total growth. | February 26. | March 6. |
|---|---|---|---|---|---|---|---|
| *Months.* | | | | | | | |
| 1 | 0 | 0 | 2.9 | 6.6 | 9.5 | | |
| 2 | 0 | 0 | 3.8 | 6.0 | 9.3 | 11.4 | 13.5 |
| 3 | 0 | 0 | 3.5 | 5.6 | 8.6 | 10.8 | 13.3 |
| 4 | 0 | 0 | 2.5 | 5.3 | 8.9 | 11.1 | 13.5 |
| 5 | 0 | 0 | 2.5 | 3.8 | 9.0 | 10.8 | 12.6 |
| Average | 0 | 0 | 3.0 | 3.6 | [b]8.8 | 11.0 | 13.2 |
| FERTILIZED.[a] | | | | | | | |
| 1 | 0 | 0 | 3.1 | 6.1 | 9.0 | | |
| 2 | 0 | 0 | 3.6 | 7.0 | 10.8 | 13.1 | 15.8 |
| 3 | 0 | 0 | 2.9 | 6.3 | 10.6 | 12.3 | 17.0 |
| 4 | 0 | 0 | 2.9 | 6.1 | 9.8 | 14.1 | 16.0 |
| 5 | 0 | 0 | 3.3 | 6.5 | 9.5 | 12.9 | 17.2 |
| Average | 0 | 0 | 3.2 | 6.4 | [b]9.9 | 13.1 | 16.5 |

[a] For every month, the average of twelve measurements was taken.
[b] Average of sixty measurements.

TABLE 5.—*Average growth per week of the unfertilized and fertilized stools at different ages in the field experiments*—Continued.

UNFERTILIZED. [a]

| Age. | March 14. | March 22 total growth. | March 29. | April 6. | April 14. | April 22 total growth. | April 29. |
|---|---|---|---|---|---|---|---|
| *Months.* | | | | | | | |
| 1 | | | | | | | |
| 2 | 15.7 | 18.2 | | | | | |
| 3 | 16.0 | 18.8 | 20.6 | 22.5 | 24.5 | 29.7 | |
| 4 | 15.9 | 19.4 | 21.7 | 24.3 | 26.9 | 32.3 | 44.7 |
| 5 | 14.8 | 17.1 | 19.7 | 23.3 | 25.3 | 31.1 | 44.9 |
| Average | 15.6 | [c] 18.3 | 20.6 | 23.3 | 25.5 | [d] 31.0 | 44.8 |

FERTILIZED. [a]

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | | | | | | | |
| 2 | 18.7 | 21.4 | | | | | |
| 3 | 20.8 | 25.4 | 30.0 | 31.0 | 41.8 | 46.0 | |
| 4 | 20.4 | 25.9 | 28.9 | 30.0 | 37.4 | 43.4 | 60.6 |
| 5 | 21.0 | 26.1 | 29.2 | 32.0 | 35.2 | 42.2 | 59.1 |
| Average | 20.2 | [c] 24.7 | 29.3 | 31.0 | 38.1 | [d] 43.3 | 59.8 |

UNFERTILIZED. [a]

| Age. | May 6. | May 14. | May 22 total growth. | May 30. | June 6. | June 14. | June 22 total growth. |
|---|---|---|---|---|---|---|---|
| *Months.* | | | | | | | |
| 1 | | | | | | | |
| 2 | | | | | | | |
| 3 | | | | | | | |
| 4 | 53.4 | 74.7 | 93.5 | | | | |
| 5 | 57.2 | 79.2 | 98.8 | 117.4 | 135.6 | 153.2 | 174.9 |
| Average | 55.3 | 76.9 | [e] 96.1 | 117.4 | 135.6 | 153.2 | [f] 174.9 |

FERTILIZED. [a]

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | | | | | | | |
| 2 | | | | | | | |
| 3 | | | | | | | |
| 4 | 73.3 | 107.6 | 140.8 | | | | |
| 5 | 77.1 | 110.0 | 130.5 | 155.6 | 170.9 | 205.7 | 239.3 |
| Average | 75.2 | 108.8 | [e] 135.6 | 155.6 | 170.9 | 205.7 | [f] 239.3 |

[a] For every month, the average of twelve measurements was taken.
[c] Average of forty-eight measurements.
[d] Average of thirty-six measurements.
[e] Average of twenty-four measurements.
[f] Average of twelve measurements.

TABLE 6.—*Weights per stool of shoots and roots and the ratio of tops to roots in the field experiment.*

| Treatment and age. | Average growth of shoots per stool. | Average weight of shoots per stool each month. | Average weight of roots per stool each month. | Ratio of tops to roots. |
|---|---|---|---|---|
| ONE MONTH | *cm.* | *g.* | *g.* | |
| Fertilized | 9.9 | 13.9300 | 1.7833 | 7.9 |
| Unfertilized | 8.8 | 12.6665 | 1.6450 | 7.7 |
| Increase over control | 1.1 | 1.2635 | 0.1183 | |
| Do per cent | 12.5 | 9.9 | 7.2 | |
| TWO MONTHS | | | | |
| Fertilized | 24.7 | 22.1504 | 2.9933 | 7.4 |
| Unfertilized | 18.3 | 18.4842 | 2.6406 | 7.0 |
| Increase over control | 6.4 | 3.6662 | 0.3527 | |
| Do per cent | 34.9 | 19.8 | 13.4 | |
| THREE MONTHS | | | | |
| Fertilized | 43.3 | 67.6269 | 9.6010 | 6.9 |
| Unfertilized | 31.0 | 50.0473 | 7.8199 | 6.4 |
| Increase over control | 12.3 | 17.5796 | 1.9811 | |
| Do per cent | 25.3 | 35.12 | 39.6 | |
| FOUR MONTHS | | | | |
| Fertilized | 135.6 | 205.5280 | 32.6235 | 6.3 |
| Unfertilized | 96.1 | 151.6992 | 25.2832 | 6.0 |
| Increase over control | 39.5 | 53.8288 | 7.3403 | |
| Do per cent | 41.1 | 35.48 | 29.0 | |
| FIVE MONTHS | | | | |
| Fertilized | 239.3 | 311.2217 | 48.6284 | 6.4 |
| Unfertilized | 174.9 | 216.1004 | 35.4263 | 6.1 |
| Increase over control | 64.4 | 95.1213 | 13.4263 | |
| Do per cent | 36.9 | 44.00 | 37.2 | |

## DISCUSSION OF THE RESULTS

*The box experiments.*—The percentages of roots in the different levels shown by Table 1 are in general in agreement with the results obtained by other investigators, in that large percentages of the roots of the cane are on the topmost surface of the soil. However, as the roots develop there is a rapid increase in the root masses in all levels, particularly the uppermost three layers. As is to be expected, as the roots grow deeper, the percentages in the topmost 6-inch layer decrease.

Comparing the fertilized with the unfertilized stools, it is seen that the root masses in practically all the layers and during

the whole period of this experiment have been higher for the fertilized than for the unfertilized stools.

Table 2 shows the growth measurements of the fertilized and unfertilized stools. The figures in Table 2 are visualized in fig. 3. It is seen here that the rate of growth of the fertilized stools is consistently greater than that of the unfertilized, and that there is a parallelism between the greater rate of growth of both the roots and the shoots of fertilized stools as compared with that of the unfertilized. We may say, therefore, that the fertilizer accelerated the growth of both the roots and the shoots of the young canes. A corresponding parallelism exists between the accelerated growth of the roots and shoots of fertilized cane and between the weights of roots and weights of the stalks. This is shown by Table 3. Table 3 also gives the ratio for shoot to root weights. The ratios for the fertilized stools are consistently higher than those for the unfertilized. This shows that the acceleration given by the fertilizer has a more pronounced effect on the shoots than on the roots.

Another noteworthy fact recorded in Table 3 is that as the cane grows, from planting up to five months, the ratio of tops to roots decreases, showing a more rapid increase in the weights of the roots during this period. This is true of both the fertilized and the unfertilized stools, and is evidently a habit of the cane plant. It is also to be noted that the drop in the ratio of tops to roots is not along a straight line but along a parabolic curve, the ratio probably falling between 5.5 and 5.8. What the ratio of roots to shoots is in mature cane only further experiments will show. The ratio will most likely again increase, and greatly pass the ratio at the start, as the cane continues its development.

The root system of Mauritius 1900 grown in the modified boxes is shown by the series of photographs given in Plates 1 to 5. Plate 1 is for a 1-month cane, Plate 2, for a 2-month cane, Plate 3, for a 3-month cane, Plate 4, for a 4-month cane, and Plate 5, for a 5-month cane. In general, the pictures show a greater mass of roots for the fertilized than for the unfertilized, and a general tendency for the roots to grow downward, in the majority of cases almost vertically.

*The field experiment.*—Table 4 shows the results of excavation of the stools in the field experiment. These results are visualized in figs. 7 and 11.

In general, the distribution of the roots in the field experiment offers the same picture as that in the box experiment.

The root system consists of stems growing from the foot of the cane and radiating in all directions but mostly downward.

Dividing the space occupied by the roots into blocks 12.5 by 12.5 by 15 centimeters and dividing the weight per section by the number of blocks in each section, we obtain figures which we may designate as densities of the root in each block. By referring to Table 4*a* it can be seen that the densities become less and less as the distances from the foot become greater. The greatest densities are in the A to A–3 sections where by far the greatest masses of roots are found. Follow these in the B, C, D sections in the order named. It is also to be noted that the density in the A-section for the 5-month-old cane is about twenty times that in the B and about seven times that in the $A_1$.

Comparing the unfertilized with the fertilized it is seen that the fertilizer increased the root densities in all blocks but that the greatest increase is in the A–$A_1$ zone, where the fertilizer was applied.

Table 5 gives the growth measurements for both the fertilized and unfertilized stools of the field experiment. As in the case of the box experiment the fertilizer is seen here to have greatly accelerated the growth of the cane.

Table 6 is a comparison of the rate of growth, weight of shoots, weight of roots, and ratio of shoots to roots, both for the unfertilized and the fertilized stools. As in the box experiments the ratios of top to roots are consistently greater for the fertilized than for the unfertilized and decrease with the age of the cane up to 5 months. The same explanation is offered that was given in the discussion of the box experiment. It is to be noted, however, that the ratios in the field experiment, both for the fertilized and the unfertilized canes, are consistently higher than those for the box experiment.

In spite of the fact that the soil in the field experiment is richer than that in the box experiment, if we may follow the general criterion that the hillocks have poorer soil than the lower flat ground, the corresponding weights of both the roots and shoots, both fertilized and unfertilized, as well as the rate of growth of the cane in the box experiment are consistently higher than in the field experiment. This difference in favor of the poorer soil is undoubtedly due to the better aëration, induced by the mechanical disturbances brought about in digging the holes, and to the watering of the soil in the boxes when returning the different layers of soil to their respective places. It is also

to be noted that in the box experiment the penetration of the roots to the lower levels is greater than in the field experiment. In other words we may have here evidence of the good effect of subsoiling in inducing downward development of the root system. Also, corresponding to the greater weight of roots in the box experiments, there is increased weight of shoots, amounting to more than 100 per cent of the weight of the shoots in the field experiment, clearly showing a correlation between shoot weights and root weights during the early life of the cane plant.

*Practical significance of the results obtained in these studies.*—The field study reported here has given us a much better picture of the root system of Mauritius variety of sugar cane, and presumably its habit is shared by the other noble varieties. The influence of aëration and fertilization is to increase the mass and distribution of the roots, but the habit of concentrating the roots in a zone below the foot, where the density of the roots is many times that of the outer zone persists throughout. The A zone, where some 80 per cent of the roots are found, is confined to the space 12.5 centimeters around the foot and to a depth of 24 inches from the surface of the soil. This must, therefore, be the zone where fertilizer should be applied, and which should be reached by the irrigation water (where irrigation is practiced).

Where to apply the fertilizer is shown in fig. 12. The best place to apply the fertilizer is indicated by the arrow. As in

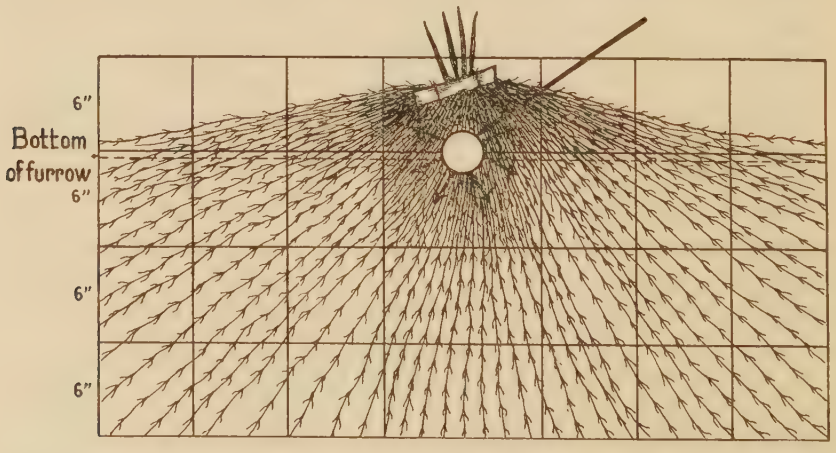


FIG. 12. Showing the most desirable place to apply the fertilizer. The long arrow indicates where the heap of fertilizer must be placed at the time of planting. If a little water is poured over it, the dissolved fertilizer will diffuse downward and spread within the space that will be occupied by the greatest mass of active roots as indicated by the small arrows.

Luzon we have good reasons for applying the fertilizer at the time of planting, the detail of application should be as follows:

1. Make deep furrows for planting.

2. Place the fertilizer deep in the furrow, approximately where the points will be planted, mix it with the soil, and pour a little water on it to carry the fertilizer down, as indicated by the small arrows in fig. 12. Then place the point in such a way that it occupies just the space above where the fertilizer was placed.

Judging from the results of this year's field tests on the methods of applying fertilizer, which we have reported in another paper, one cannot be too careful in the application of the fertilizer. A great deal may be lost with the wrong application. Even if the present cost of application were doubled, the gains to be obtained from the proper application will compensate such an additional expense from ten to twenty times over.

A second corollary to the results of these root studies is in the method of cultivation. The distribution of the roots of the cane is such that plowing within 5 inches from the foot of the cane is to be decried. Cultivators that penetrate more than 3 inches must be passed some 6 inches away from the stools. When such cultivators are thus used, they will destroy insignificant amounts of root. On the other hand, if a plow is passed to within 5 inches of the foot and to a depth of 6 inches, as much as 7 per cent of the roots may be cut off, and such pruning will undoubtedly result in the stunting of the cane. The difference in root masses between the 5-month-old unfertilized and the 5-month-old fertilized cane is around 37.2 per cent of the weight of roots of the unfertilized cane. This difference in root mass is responsible for a difference of 44 per cent in the weight of the tops in favor of the fertilized canes. Pruning 7 per cent of the roots of the cane, unless the variety has great recuperative power, cannot but have a retarding effect on the rate of growth of the cane.

The present studies were limited to five months because the junior author who executed them had only that much time to spend at the station, since at the end of that period he had to return to college. So it was decided to limit our preliminary studies to the period of cultivation of the cane which is the critical period of growth, and the time when the size of the crop may be greatly affected by proper or improper cultivation methods. After five months, in June, the cane has usually closed up, and receives no further attention until harvest time. In other

words, from the point of view of human influence on the size of the crop, this should be considered the most important period. After this period, the fate of the cane is largely dependent on the monsoons. However, the results obtained in this preliminary work encourages us to plan more extensive studies to include other varieties, and the whole period of the life of the cane plant.

A suggested method of studying roots in relation to cultural methods is to divide the root zones as indicated in fig. 13.

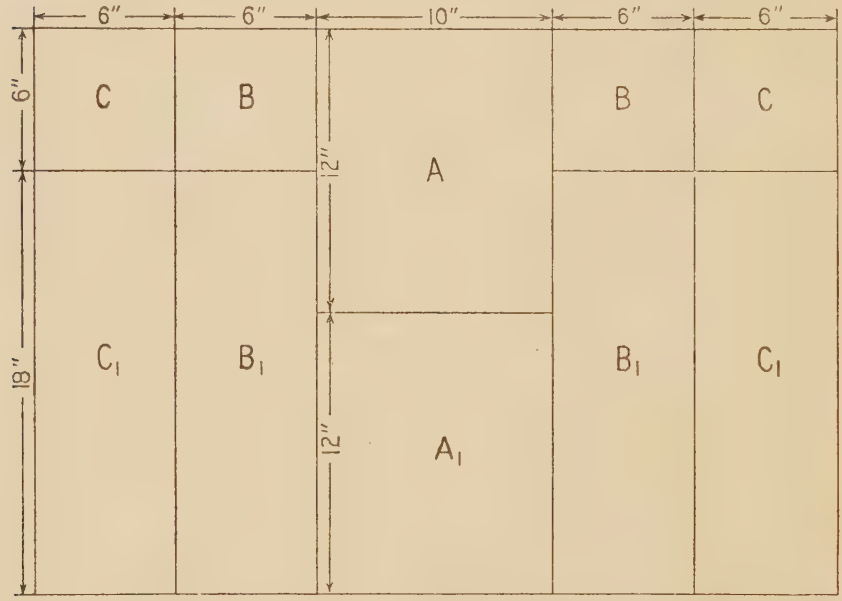


FIG. 13. Showing the proposed zones to be excavated in studying the root systems of different varieties and the effect of cultural treatments on their roots development.

Standard figures for five-month canes may be established for the different zones for different soil conditions and varieties. If in excavations made in commercial plantings it is discovered that the root mass in any of these zones is below standard, reason for it must be sought.

## SUMMARY OF CONCLUSIONS

1. Two series of studies on the root and shoot development of Mauritius 1900 variety of cane were conducted. In one, a modified-box method was used, in which wire netting held on frames nailed on posts was placed in holes in the ground and

the subsoil and soil layers were replaced in their original condition. In the other, the cane was planted in replicated plots, and arranged in *dama-dama*. Two treatments were used—with and without fertilizer.

2. In both studies it was found that increased shoot development corresponded to increased root development, without exception.

3. The roots of the cane develop faster than the shoot during the first months of its growth. This is in line with the old conception that plants first develop a good root system before shoot development will take place actively.

4. Aëration of the subsoil favors the formation of greater masses of roots in the lower levels.

5. The cane plant tends to concentrate its mass of roots within a zone about 5 inches on each side and 12 to 18 inches below the foot of the cane. This is the zone where fertilizer must be applied.

6. Fertilizer increased the masses of roots through the whole system and not only in the zone where the fertilizer was applied.

7. The suggested method of application is to place the fertilizer in the furrow just before planting in an oval of about 6 inches, mixing it thoroughly with the soil and pouring water over it to carry it down to the lower levels. The point should be planted over this oval.

8. Further studies, using the method followed in this paper and including other varieties and the whole period of growth of the cane plant, are recommended.

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# ILLUSTRATIONS

ROOT SYSTEMS OF MAURITIUS 1900 GROWN IN MODIFIED BOXES.

PLATE 1. A 1-month cane.
2. A 2-month cane.
3. A 3-month cane.
4. A 4-month cane.
5. A 5-month cane.

TEXT FIGURES

FIG. 1. Graph showing the arrangement of the modified-box experiment.
2. Graph showing the weight of roots in the different horizons and at different ages both for the fertilized and unfertilized cane in the box experiment.
3. Graph comparing growth, weight of shoots, and weight of roots of the fertilized and the unfertilized stools in the modified-box experiment.
4. Graph showing the distribution in percentages of the total weight of the roots in the different horizons of M–1900 in the modified-box experiment.
5. Graph showing the arrangement of plots in the experimental field.
6. Graph showing the different sections of the excavation.
7. Graph showing the weight of roots of both the unfertilized and fertilized stools in the different sections at different levels and ages under field conditions.
8. Graph comparing the total growth in centimeters, weight of shoots, and weight of roots of both the unfertilized and the fertilized stools at different ages under field conditions.
9. Graph showing the weight of roots of both the fertilized and unfertilized stools at different depths and ages in the field experiment.
10. Graph showing the distribution in percentages of the total weight of the roots in the different levels of M–1900 under field conditions.
11. Graph showing in figures the weights and percentages of roots in the different sections, and in drawings a section of the root system of M–1900, as reconstructed from its quantitative distribution.
12. Graph showing the most desirable place to apply the fertilizer. The long arrow indicates where the heap of fertilizer must be placed at the time of planting. If a little water is poured over it, the dissolved fertilizer will diffuse downward and spread within the space that will be occupied by the greatest mass of active roots as indicated by the small arrows.
13. Graph showing the proposed zones to be excavated in studying the root systems of different varieties and the effect of cultural treatments on their root development.

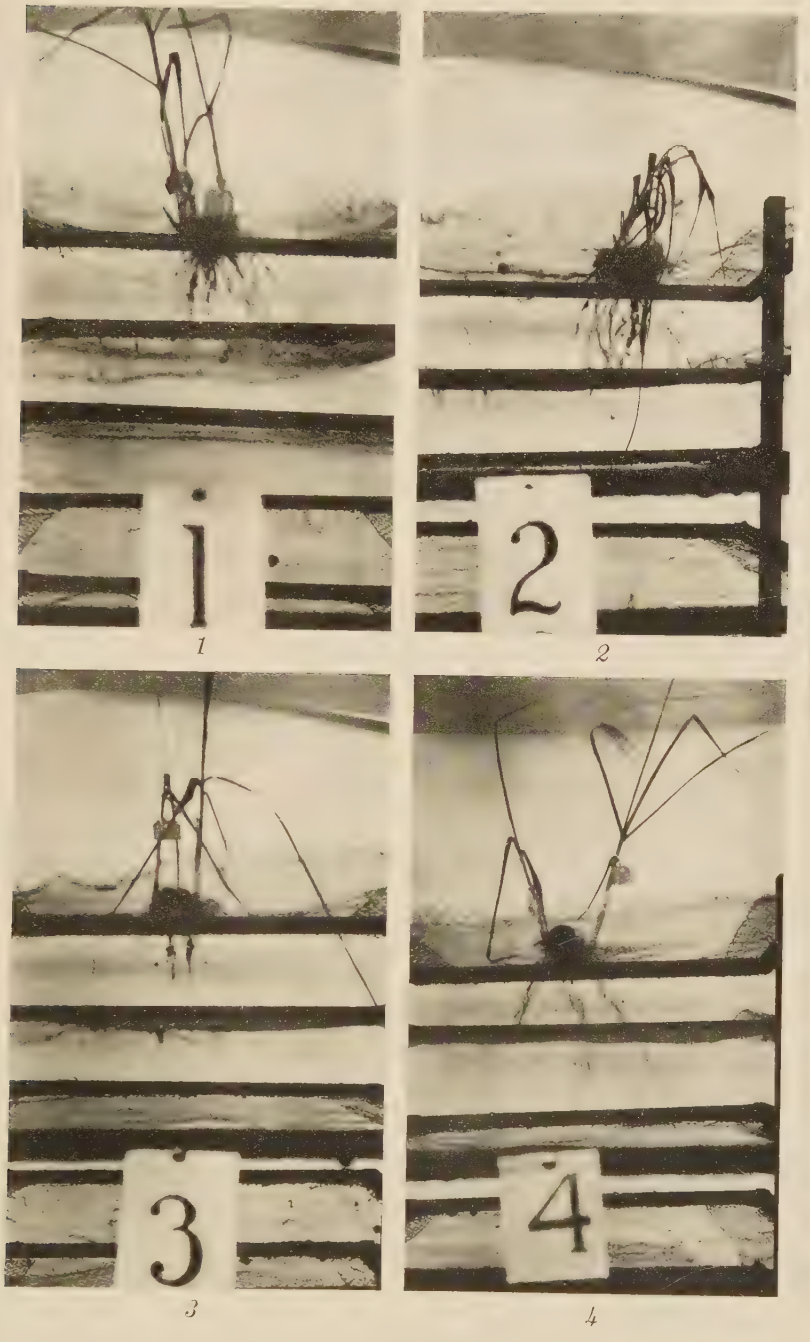


PLATE 1.

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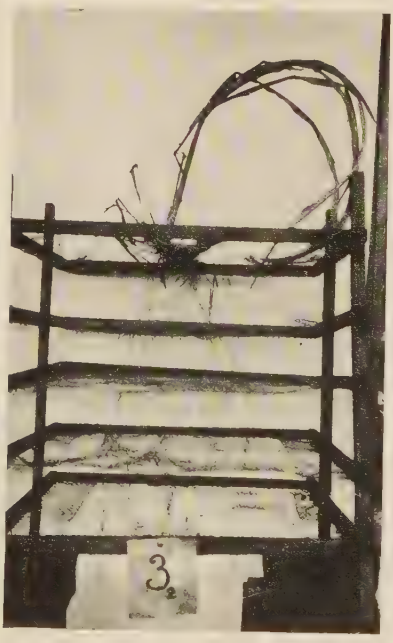


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PLATE 2.

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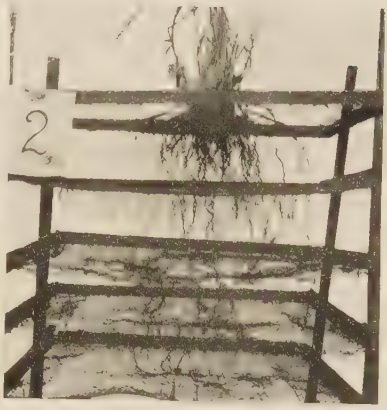


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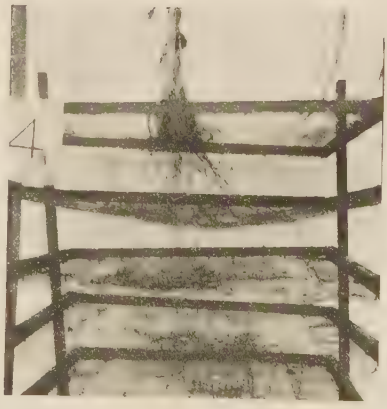


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PLATE 3.

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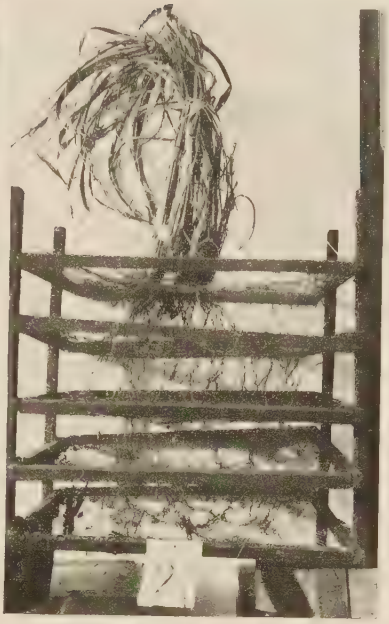

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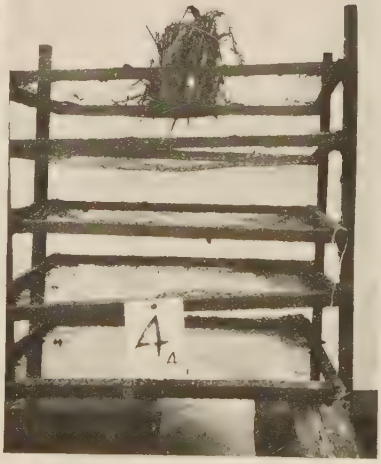


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PLATE 4.

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PLATE 5.

# THE OCCLUSION OF LEAD AND COPPER IN NONFERROUS ALLOYS BY METASTANNIC AND METANTIMONIC ACIDS

By Salvador del Mundo

*Chemist, Division of Inorganic Chemistry, Bureau of Science, Manila*

In the analysis of alloys containing tin with or without antimony, it is customary to separate the tin, and the antimony if present, by nitric acid attack whereby these metals are precipitated as metastannic and metantimonic acids, respectively. If lead, phosphorus, copper, and iron are present, considerable quantities of these metals are adsorbed and dragged down with the precipitated metastannic and metantimonic acids, wherefore the usual procedures call for the purification of the ignited precipitate by fusion with six times its weight of a mixture of equal parts of sodium carbonate and pure sulphur.[1] This process of purification is not only tedious and cumbersome, but also requires considerable practice and skill if reliable results are to be expected. Therefore, it is often preferred to avoid attack by nitric acid. However, there are advantages to be gained in the early separation of tin and antimony in the analysis of nonferrous alloys, so that if the extent of adsorption were known, it would seem that valuable data could be furnished the analyst.

In this laboratory, experience in the analysis of nonferrous alloys has pointed out a number of interesting results on the adsorption of lead, chiefly in white metals and copper in bronze by precipitated metastannic and metantimonic acids.

In one set of experiments, white metals containing tin and antimony in a lead base were used. One alloy was of the following composition:

| Constituent. | Per cent. |
|---|---|
| Lead (Pb) | 82.81 |
| Tin (Sn) | 4.49 |
| Antimony (Sb) | 12.03 |
| Iron (Fe) | 0.10 |
| Copper (Cu) | 0.05 |
| Arsenic (As) | trace |

[1] Treadwell and Hall, Analytical Chemistry, 6th ed. John Wiley and Sons, New York 2 (1924) 215.

For technical purposes, iron, copper, and arsenic may be regarded as impurities and the alloy considered to have a Sn: Sb: Pb ratio of 5: 12: 83.

One-half gram of the finely divided alloy was treated with 5 cubic centimeters of concentrated nitric acid (specific gravity, 1.4) followed by 10 cubic centimeters of water. The mixture was placed on a hot plate and when violent action ceased, it was boiled until no more red fumes were given off and the alloy was completely decomposed. The mixture was then transferred to a steam bath where it was evaporated to dryness and the residue baked for about thirty minutes, after which it was taken up with 5 cubic centimeters of concentrated nitric acid and diluted with 50 cubic centimeters of water. The mixture was left on the steam bath and the precipitated substances allowed to settle completely. The precipitate was then filtered and washed alternately with a 2 per cent solution of nitric acid and hot water. Lead was determined in the filtrate by the usual method of conversion into sulphate, followed by extraction with hot acid ammonium acetate and precipitation of lead chromate from the boiling hot acetate extract. The amount of lead thus obtained by duplicate determinations was found to be 80.15 per cent, which is lower than the true value by 2.66 per cent.

As it was thought that possibly the process of boiling the mixture of nitric acid and alloy and subsequent baking of the precipitated oxides might have caused excessive occlusion, the experiment was repeated with the following modifications. The alloy after treatment with nitric acid was heated just so as to expel all red fumes and promote complete digestion, but under no circumstances was the mixture allowed to boil. When the sample was completely decomposed, it was diluted with 50 cubic centimeters of water without any previous evaporation to dryness or baking on the steam bath. The precipitate in the diluted mixture was allowed to settle and then filtered out. When lead was determined in the filtrate in the usual manner, the percentage obtained was 81.48, which is 1.33 per cent lower than the true value. This result was verified by Pedro G. Rivera, of the Bureau of Science. It can be seen that the quantity of lead adsorbed was reduced by the elimination of boiling and baking. When the ignited oxides of tin and antimony were fused with sodium carbonate and sulphur, the quantity of lead occluded was found by electrolysis to be 1.26 per cent, which agrees with the shortage of 1.33 per cent of lead in the filtrate.

The experiments were repeated with another lead-base white metal of the following composition:

| Constituent. | Per cent. |
|---|---|
| Lead (Pb) | 85.33 |
| Tin (Sn) | 4.12 |
| Antimony (Sb) | 10.41 |
| Copper and iron | Trace. |

This alloy has a Sn : Sb : Pb ratio of 4 : 11 : 85.

When treated with nitric acid, boiled, and the precipitated oxides of tin and antimony baked, the lead found in the nitric acid filtrate was 82.69 per cent as against 85.33 per cent, which is the true value. The shortage of 2.64 per cent in lead, which is the quantity occluded by the combined oxides, confirms the results of the preceding experiments. When no boiling was resorted to in the decomposition of the alloy and in the expulsion of red nitric fumes, and when the precipitated oxides were not baked, the amount of lead found in the nitric acid filtrate was 84.56 per cent. This value is lower than the true lead content by 0.77 per cent. The quantity of lead occluded is thus seen to be nearly 1 per cent.

From these results, it will be noted that in lead-base alloys containing tin and antimony, the quantity of lead adsorbed varies from 1 to 3 per cent depending on the method of attack and the treatment of the precipitated oxides. Whereas, the practice of baking the precipitated metastannic and metantimonic acids renders the process of filtration easier, still in view of the above results, it does not seem to be practical when due account is taken of the resulting increase in occlusion. It is of interest to note that for a given method of procedure, the quantity of lead occluded is fairly constant. This remark is of special interest, as it might be expected that the quantity of lead occluded would vary from determination to determination.

In view of the results of the foregoing experiments, it is difficult to understand how T. B. Diana [2] has come to the conclusion that in the routine analysis of tin in lead-base alloys containing antimony "the correction for PbO may be neglected." As a matter of fact, Griffin,[3] who gives exactly the same procedure as the one proposed by Diana, prescribes the purification of the ignited oxides of tin and antimony.

[2] The Chemist Analyst 18 (1929) 8.

[3] Technical Methods of Analysis, 2d ed. McGraw Hill Book Co., Inc., New York (1927) 196.

In the case of Babbitt alloys which are high in tin, the quantities of lead and copper occluded by precipitated metastannic and metantimonic acids have been found to vary from 0.1 to 0.5 per cent, seldom if ever higher than 1 per cent. In the case of brass or bronze, this laboratory has confirmed the statement of Griffin,[4] who says that for alloys, "containing less than 10 per cent Sn and less than 0.7 per cent P, no further purification of the mixed oxides is necessary; but if above these limits, the mixed oxides must be purified," by fusion with sodium carbonate and pure sulphur.

Should it be desired to determine tin directly in nonferrous alloys, apparently the best method for technical purposes is that based on iodimetric titration.[5] For the direct determination of antimony, this laboratory uses the method based on the oxidation of trivalent to quinquevalent antimony in sulphuric-hydrochloric acid solution by means of standard potassium permanganate. For a direct determination of lead in alloys containing tin and antimony in a lead base, this laboratory has worked out a method which gives satisfactory results. The process consists of a simultaneous decomposition of the alloy and precipitation of all the lead as sulphate by means of concentrated sulphuric acid. Tin and antimony are held in solution by proper acid concentration. Details of the method are as follows.

One-half gram of fine hack-saw filings is treated with 10 cubic centimeters of concentrated sulphuric acid and heated until the alloy is completely decomposed and all the separated sulphur driven off. The liquid is cooled and then very carefully diluted with 50 cubic centimeters of water. The precipitate of lead sulphate is allowed to settle completely and then filtered and washed several times by decantation with a 5 per cent solution of sulphuric acid followed by cool water. As the lead sulphate precipitate may be contaminated with small amounts of basic salts of tin and antimony, as well as with traces of precipitated sulphur, it is not determined as such. It is extracted with hot acid ammonium acetate and determined as lead chromate in the usual manner.

This method has been found to be efficient in the direct determination of lead in white metals made on a lead base. It affords

[4] Technical Methods of Analysis, 2d ed. (1927) 181.

[5] Hillebrand and Lundell, Applied Inorganic Analysis. John Wiley and Sons, New York (1929) 237. U. S. Bureau of Standards Certificate of Analyses of Standard Samples Nos. 53 and 54.

a fairly clean separation of lead from tin and antimony without loss due to occlusion or solution. It gives concordant results with those of the method based on the solution of the alloy with nitric acid and subsequent recovery of lead occluded in the combined oxides. It also agrees with the method based on the solution of the alloy with tartaric and nitric acids followed by precipitation of lead, as sulphate. Moreover, it possesses the advantage of being rapid and sufficiently accurate for routine analyses. While it might seem as though the decomposition of the alloy with concentrated sulphuric acid would be difficult, this is not the case. When the mixture is heated on a hot plate or on a Bunsen flame, a completely decomposed product is obtained in from twenty to thirty minutes. The presence of sulphuric acid prevents the precipitation of basic tin and antimony salts, and even if small quantities of such basic salts were to separate with the precipitated lead sulphate no harmful effects would result, since the subsequent extraction with ammonium acetate eliminates such impurities. For alloys high in tin, this method is not recommended as it is then not possible to avoid the precipitation of basic salts of tin and antimony in quantities large enough to cause complications.

## SUMMARY

A study is presented in this paper on the adsorption and occlusion of lead by metastannic and metantimonic acids, formed by nitric acid attack on nonferrous alloys. In white metals containing tin and antimony in a lead base, the quantity of lead occluded varies from 1 to 3 per cent, a value that is too high to be disregarded as negligible. In alloys high in tin, however, the quantity of lead and copper that may be occluded varies from 0.1 to 0.5 per cent. For a given alloy and a given method of attack, the quantity of lead occluded is constant.

A satisfactory method has been worked out for the direct determination of lead in white metals containing tin and antimony in a lead base.

# NAPHTHOL ESTERS OF CHAULMOOGRIC ACID AND CHAULMOOGRYL NAPHTHYLAMINES

By Irene de Santos and Augustus P. West

*Of the Bureau of Science, Manila*

Various derivatives of chaulmoogric acid have been made from chaulmoogra oil. In the present investigation the naphthol esters of chaulmoogric acid were prepared as well as the chaulmoogryl naphthylamines. The naphthol esters were made by treating the acid chloride of chaulmoogric acid with the naphthols, while the chaulmoogryl naphthylamines were obtained by treating the acid amide of chaulmoogric acid with the naphthylamines. These new compounds thus prepared will be tested for their therapeutic value. In order to check the formulas of the chaulmoogryl naphthylamines, the nitrogen content was determined. A modification of Meulen's catalytic method [1] was employed for making the nitrogen analyses.

## EXPERIMENTAL PROCEDURE

The chaulmoogra oil used in this investigation was kindly presented to us by Dr. H. I. Cole, of the Philippine Bureau of Health, and was shipped directly to us from the Culion Leper Colony. The oil was prepared from the seeds of *Hydnocarpus alcalæ* C. de Candolle. This oil contains about 90 per cent of chaulmoogric acid.[2] Since the chaulmoogric acid content of this oil is unusually high it naturally serves as a good source of material for the preparation of chaulmoogric acid and its derivatives and also chaulmoogryl substituted compounds.[3]

The chaulmoogric acid, acid chloride, and acid amide of chaulmoogric acid were prepared according to the procedure of San-

[1] Smith, F. L., and A. P. West, Philip. Journ. Sci. 31 (1926) 265.

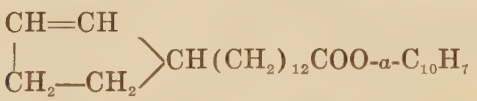
[2] Brill, H. C., Philip. Journ. Sci. § A 12 (1917) 37.

[3] Herrera-Batteke, P. P., and A. P. West, Philip. Journ. Sci. 31 (1926) 161. Santiago, S., and A. P. West, Philip. Journ. Sci. 33 (1927) 265; 35 (1928) 405. Santillan, P., and A. P. West, Philip. Journ. Sci. 40 (1929) 493. De Santos, I., and A. P. West, Philip. Journ. Sci. 38 (1929) 293 and 445; 40 (1929) 485; 41 (1930) 373.

tiago and West.[4] Chaulmoogra oil was saponified with alcoholic potassium hydroxide. The residual soaps were decomposed with dilute sulphuric acid and the free acids extracted with ether. The ether extract was dehydrated with anhydrous sodium sulphate and filtered, after which the solution was distilled to eliminate the ether. The residue was treated with gasoline, and the precipitated resin acids were separated by filtering. The solution was evaporated somewhat and allowed to crystallize. The crude product was recrystallized several times from alcohol (95 per cent). The melting point of the purified chaulmoogric acid was 68° C.

The acid chloride of chaulmoogric acid was prepared by treating melted chaulmoogric acid with phosphorus trichloride. The reaction was finished in about fifteen minutes. The reaction product was filtered through glass wool to remove the viscous phosphorous acid, and the clear filtrate consisting of the acid chloride of chaulmoogric acid was allowed to drop slowly into cold concentrated ammonia. The precipitated amide was washed with water and dried. The amide was crystallized from methyl alcohol and also from xylene. The crude product was then dissolved in absolute alcohol, the solution decolorized with vegetable carbon (suchar), and crystallized. The melting point of the amide was 104 to 105° C.

α-NAPHTHOL ESTER OF CHAULMOOGRIC ACID



In the preparation of this compound 20 grams of chaulmoogric acid were treated with 2.3 cubic centimeters of phosphorus trichloride. The acid chloride of chaulmoogric acid, thus obtained, was then mixed with 10 grams of α-naphthol. The flask containing the materials was heated in an oil bath (Crisco) at a temperature of about 100° C., until no more acid vapors were evolved. This required about eight days. The reaction product was a dark brown oil. This was poured into water and extracted with ether. The ether extract was then washed with water until free of acid. The extract was then dehydrated with anhydrous sodium sulphate, filtered and distilled to remove the ether. The residue, which was a thick dark-colored oil, was then distilled under reduced pressure. A small amount of liquid distilled over at 180° C., under 14 millimeters pressure. The

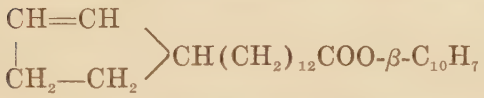
[4] Philip. Journ. Sci. 33 (1927) 265.

residue in the flask was dissolved in methyl alcohol and decolorized twice with vegetable charcoal (suchar). Traces of suchar that remained in the solution were removed by treating with talcum powder and filtering. The solution was then evaporated somewhat and allowed to crystallize. When recrystallized from ethyl alcohol, slightly yellowish crystals melting at 53 to 54.5° C., were obtained. The yield was about 10 per cent. The α-naphthol ester of chaulmoogric acid was found to be soluble in the following solvents: Ethyl alcohol, acetone, amyl alcohol, chloroform, ether, carbon bisulphide, petroleum ether, ethyl acetate, ethyl benzoate, and propyl alcohol.

Analysis:

| | Carbon. Per cent. | Hydrogen. Per cent. |
|---|---|---|
| Calculated for $C_{28}H_{38}O_2$ | 82.70 | 9.43 |
| Found | 82.58 | 9.59 |

β-NAPHTHOL ESTER OF CHAULMOOGRIC ACID

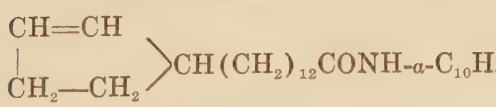


The chaulmoogric acid (20 grams) was converted into the acid chloride, which was then treated with 10 grams of β-naphthol. The mixture was heated in a Crisco oil bath (125° C.) until no more acid vapors were evolved. This required about six days. The reaction product, which was a dark brown solid, was dissolved in methyl alcohol and decolorized three times by treating with suchar. After removing traces of suchar with talcum powder the solution was allowed to evaporate somewhat and crystallize. White crystals, which melted at 49.5 to 51° C., were obtained. The yield was about 50 per cent. The β-naphthol ester of chaulmoogric acid dissolves readily in the following cold solvents: Ethyl alcohol, acetone, amyl alcohol, chloroform, ether, carbon bisulphide, ethyl acetate, ethyl benzoate, xylene, and propyl alcohol.

Analysis:

| | Carbon. Per cent. | Hydrogen. Per cent. |
|---|---|---|
| Calculated for $C_{28}H_{38}O_2$ | 82.70 | 9.43 |
| Found | 82.55 | 9.76 |

CHAULMOOGRYL-α-NAPHTHYLAMINE



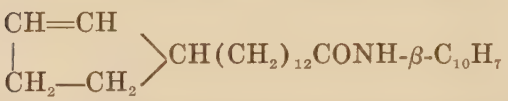
Chaulmoogric acid (20 grams) was converted into the acid chloride of chaulmoogric acid, which was then treated with

ammonia. The acid amide of chaulmoogric acid, thus prepared, was mixed with 8 grams of α-naphthylamine (Kelbe's reaction).[5] The mixture was heated in a Crisco oil bath (100° C.) until no more ammonia vapors were evolved. This required about five days. The reaction product, which was a brown solid, was dissolved in methyl alcohol. The solution was treated three times with suchar and once with talcum. When allowed to crystallize, slightly yellowish crystals were obtained. These crystals, which had a somewhat unpleasant odor, melted at 93 to 95° C. The yield was about 30 per cent. Chaulmoogryl α-naphthylamine is readily soluble in acetone, ethyl alcohol, amyl alcohol, ether, chloroform, carbon bisulphide, ethyl benzoate, ethyl acetate, xylene, and propyl alcohol.

Analysis:

| | Nitrogen. Per cent. |
|---|---|
| Calculated for $C_{28}H_{39}NO$ | 3.46 |
| Found | 3.45 |

CHAULMOOGRYL-β-NAPHTHYLAMINE



The acid amide of chaulmoogric acid, prepared from 20 grams of chaulmoogric acid, was treated with 8 grams of β-naphthylamine. The mixture was heated in a Crisco oil bath (115° C.) for a week. The reaction product was a dark-colored solid. This was dissolved in methyl alcohol and the solution treated twice with suchar and once with talcum. When evaporated somewhat, slightly yellowish crystals were obtained. The melting point was 96 to 98° C., and the yield about 40 per cent. Chaulmoogryl-β-naphthylamine dissolves readily in the following: absolute alcohol, acetone, amyl alcohol, carbon bisulphide, chloroform, ether, ethyl acetate, ethyl benzoate, propyl alcohol, and xylene.

Analysis:

| | Nitrogen. Per cent. |
|---|---|
| Calculated for $C_{28}H_{39}NO$ | 3.46 |
| Found | 3.34 |

## SUMMARY

Four compounds were prepared in this investigation; namely, two naphthol esters of chaulmoogric acid and two chaulmoogryl naphthylamines.

[5] Ber. Deut. Chem. Gesell. 16 (1883) 1199.

The naphthol esters of chaulmoogric acid were made by treating the acid chloride of chaulmoogric acid with the naphthols, while the chaulmoogryl naphthylamines were obtained by treating the acid amide of chaulmoogric acid with the naphthylamines. Our results indicate that these compounds may be prepared rather easily.

ACKNOWLEDGMENT

We wish to express our thanks and appreciation to Dr. Otto Schöbl and Miss Rita Villaamil, of the Bureau of Science, Manila, for determining the bactericidal properties of these compounds.

# NOTES ON PHILIPPINE NABIDÆ, WITH A CATALOGUE OF THE SPECIES OF GORPIS (HEMIPTERA)[1]

By HALBERT M. HARRIS

*Of Ames, Iowa*

ONE TEXT FIGURE

Through the courtesy of Dr. A. Wetmore and Dr. Harold Morrison, of the United States National Museum, a part of the Nabidæ taken by Prof. C. F. Baker in the Philippine Islands has come to me for study. This collection, while consisting of only a few species, contains some little-known forms, including four new species, and adds considerably to our understanding of Oriental nabids.

**ARISTONABIS REUTERI Bergroth.**

*Aristonabis reuteri* BERGROTH, Philip. Journ. Sci. § D 13 (1918) 117.

Specimens are at hand from Iligan (type locality) and Butuan, Mindanao; Malinao, Tayabas; Cuernos Mountains, Negros; and Samar. All are females; the male is unknown. The Iligan specimen (type?) agrees well in color with Bergroth's description except that the pronotal collar is luteous, concolorous with the anterior lobe. The other examples show the variation in color that may be expected in this species, the head, pronotum, apex of corium, abdomen, and legs being concolorously luteous in one while in another they are, respectively, concolorously sanguineous. The ratio of pronotal length to width is 33 : 52. Other proportions as given by Bergroth do not hold for the specimens before me. The pronotal width at collar (19) is slightly more than half that of anterior lobe (30), which in turn is distinctly not "twice broader than head" (18). While the size is somewhat variable, the proportions are constant. Length, 4.8 to 5.8 millimeters; width, 1.8 to 2.3.

**PHORTICUS VARIEGATUS sp. nov.**

Oblong, rather thickly clothed with semierect, fine, pale hairs; brownish, variegated with ochraceous. Head shiny, testaceous,

[1] Contribution from the Department of Zoölogy and Entomology, Iowa State College, Ames, Iowa.

paler distally, the apex almost flavous; as broad as long (male, 15 : 15; female, 17 : 17), somewhat tumid beneath. Eyes reddish, rather coarsely granular. Ocelli prominent. Antennæ flavo-testaceous, segment II slightly darker on basal two-thirds; segments I and II stout, I with its apex distinctly surpassing tip of head, II gradually enlarged distally, all rather thickly clothed with fine hairs; proportional lengths of segments, I : II : III : IV = 7 : 12 : 12 : 14. Rostrum pale testaceous, the apical segments paler; segment III somewhat swollen, proportion of segments, II : III : IV = 12 : 9 : 6.

Pronotum brown, the anterior lobe paler, with a triangular patch on the apical one-fourth ochraceous; broader than long (male, 35 : 28; female, 43 : 31), transversely impressed behind the middle, the impression bearing a row of coarse punctures; anterior lobe with a distinct median longitudinal sulcus on the disc, the sulcus deepest where it ends just before transverse depression. Scutellum concolorous with basal lobe of pronotum, the apex slightly paler; deeply foveate on the disc, the base strongly transversely depressed, the sides sinuate and provided with a row of coarse punctures along each side before the apex. Hemelytra brown, a rectangular spot at the base (extending to a point opposite middle of scutellum), a smaller, less-distinct oval spot in outer apical angle of corium, and an irregular patch on disc of corium and apex of clavus ochraceous. Membrane smoky brown, a small area joining outer apical angle of corium pale to whitish; extending to (female) or scarcely to (male) tip of abdomen. Connexivum exposed, its segments indistinct. Undersurface testaceous to brownish; legs flavotestaceous, the anterior femora except base and apex darker. Anterior femora incrassate, armed before the middle with a stout tooth and finely denticulate from there towards apex; anterior tibiæ strongly widened distally, their apices with distinct fossæ. Intermediate and posterior legs simple, pilose, unarmed. Venter thickly pilose, the segments with a row of coarse punctures along their bases. Length, male and female, 1.9 to 2.5 millimeters; width, 0.74 to 1 millimeter.

Holotype (male) and allotype (female), Mount Maquiling, Luzon, C. F. Baker, collector. Paratypes, 17 males and 5 females taken with type; 2 males and 2 females, Kolambugan, Mindanao; 1 female, Tangkulan, Bukidnon; 1 male, Mount Banahao, Luzon. Holotype and allotype in collection of the United States National Museum. Paratypes in the collections of United States National Museum and of the author.

In this beautiful little species the intensity of the brownish color varies, but the pattern is constant. The pale discal patch of the elytra occupies only the two inner cells of the corium and the apex of the clavus and is interrupted by the darker veins. The segments of the antennæ and rostrum of the female are slightly longer than in the male.

ALLOEORHYNCHUS VINULUS Stål.

*Alloeorhynchus vinulus* STÅL, Ann. Soc. Ent. France, IV 4 (1864) 59.

*Alloeorhynchus pulchellus* STÅL, Öfv. Svenska Vet. Soc. Förh. 27 (1870) 675.

*Alloeorrhynchus vinulus* REUTER et POPPIUS, Acta Soc. Sci. Fenn. 37 No. 2 (1909) 34, 37.

A nice series of this species is at hand from Los Baños and Mount Maquiling, Luzon. The writer has also seen a specimen, belonging to the Hamburg Museum, from Phuc-Son, Annam.

ALLOEORRHYNCHUS BAKERI sp. nov. Fig. 1, a.

Elongate oval, rather flat and depressed. Fuscous brown to piceous, the apical segment of rostrum, margin of abdomen along basal half, distal half of intermediate and posterior tibiæ and all trochanters and tarsi paler; hemelytra with a large oval patch on corium, extending inward to inner vein, reddish yellow. Shiny, the scutellum, hemelytra, excepting narrow costal margin, and metapleura dull. Head broader than long (51 : 45); vertex arched, its width equal to length of anteocular part of head (23 : 23); gula flat. Eyes large, as seen from the side projecting below the gula, their length from above equal to width of vertex. Ocelli prominent. Antennæ pilose, segment I extending slightly beyond apex of head; proportion of segments, I : II : III : IV = 18 : 68 : 49 : (52?). Rostrum with segment I rather horizontal, continued in same line with apex of head, II and III stout, II not reaching to base of eyes, III slender; proportion of segments, II : III : IV = 40 : 35 : 27.

Pronotum smooth, rather flat, beset with numerous long stout hairs, broader than long (105 : 78), constricted well behind the middle, the anterior lobe with a short, shallow, median, longitudinal depression before the base; posterior lobe strongly widened, its basal margin almost straight. Scutellum clothed with numerous long upright hairs, bifoveate and slightly rugose on the disc, the sides raised and somewhat sinuate, the extreme apex truncate and shiny. Hemelytra smooth, with many upright brown hairs, the sides strongly sinuate; the clavus with a double row of coarse punctures along the vein and a few punctures

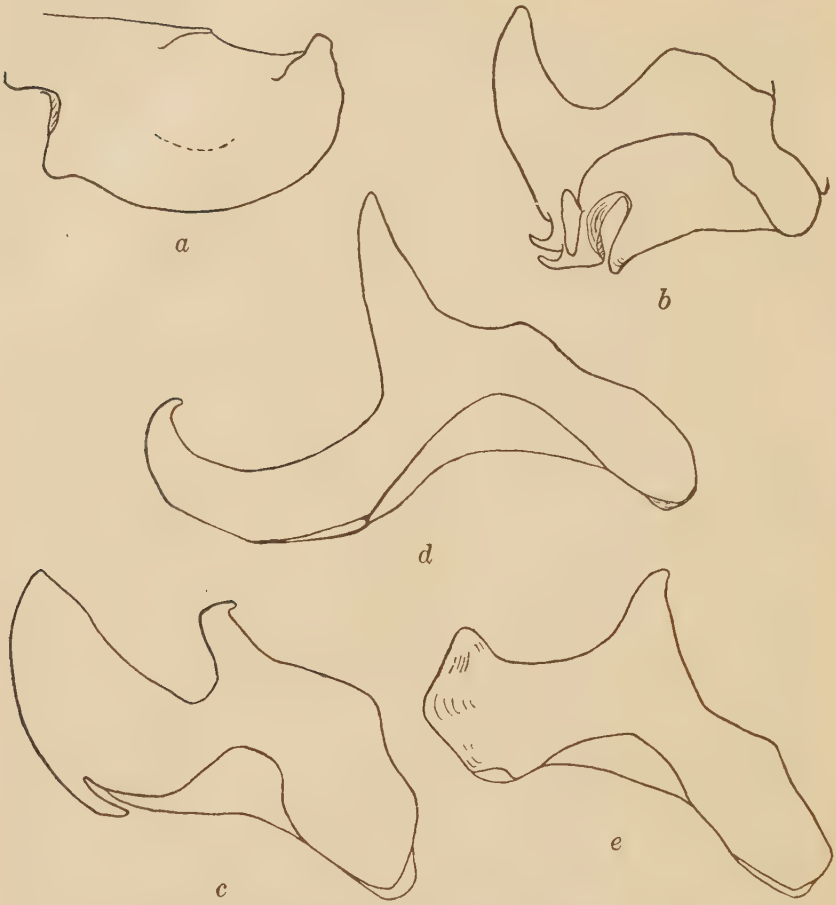



FIG. 1. Male claspers of various nabids; *a*, *Alloeorrhynchus bakeri* sp. nov.; *b*, *Nabis tagalicus* Stål; *c*, *Gorpis sordidus* Reuter; *d*, *G. flavicans* sp. nov.; *e*, *G. philippinensis* Stål.

along its inner margin next to apical half of scutellum; corium with a single row of punctures along the inner vein, its other veins obsolete; membrane smoky, slightly surpassing tip of abdomen. Mesosternum at the rear and metasternum throughout with a sharp median longitudinal carina. Metapleuron rugose, the canal shiny, its apex raised, free from pleuron and curved posteriorly. Legs thickly pilose and also setose, anterior and intermediate femora armed beneath throughout their lengths with many black spinelike teeth; anterior tibiæ strongly widened distally, serrately dentate within, the apex with a prominent spongy fossa; intermediate tibiæ faintly curved, armed within, and with a small apical pad. Venter with numerous long hairs;

the genital segment depressed on each side, the median ventral part produced into a prominent process. Clasper with broad blade (fig. 1, *e*). Length, 7.4 millimeters; width of pronotum, 2.1; width of abdomen, 2.8.

Holotype, male, Samar, Philippines, C. F. Baker, collector; in collection of the United States National Museum.

This species appertains to the subgenus *Psilistus* Stål and may be readily separated from the other members of that group by its size and coloration. The yellow patches of the hemelytra are more or less suffused and flecked with blood red. The type bears the number 23469 and the label, *Alloeorhynchus bakeri* Bergr., in Professor Baker's handwriting.

NABIS TAGALICUS Stål. Fig. 1, b.

*Nabis tagalicus* STÅL, Freg. Eug. Resa, Ins. (1859) 261.
*Nabis tagalicus* REUTER, Öfv. Vet. Akad. Förh. 29 No. 6 (1872) 88, fig. 8.
*Nabis tagalicus* REUTER, Mém. Soc. Ent. Belg. 15 (1908) 105, 107.

This species belongs to the subgenus *Stenonabis* Reuter. Numerous specimens from Los Baños and Mount Maquiling, Luzon, are present in the Baker collection. The claspers of the males are produced on the lower outer margin of their blades into three hooklike processes (fig. 1, *d*).

GORPIS SORDIDUS Reuter. Fig. 1, c.

*Gorpis sordida* REUTER, Ann. Soc. Ent. Belg. 52 (1909) 428.
*Gorpis sordidus* POPPIUS, Ann. Mus. Zool. Acad. Sci. 19 (1914) 139.

Numerous specimens of this species are at hand from the following localities: Los Baños and Mount Maquiling, Laguna Province; Imugan, Nueva Vizcaya Province; and Baguio, Mountain Province, Luzon. Northwestern Panay. Samar Island. Surigao, Zamboanga, and Butuan, Mindanao. The species originally was described from New Guinea and Deslacs Island and afterwards was recorded from Los Baños, Philippine Islands. The color markings, as shown by the series at hand, are somewhat variable, although the pattern is fairly constant. Some specimens have sordid- and nigro-fuscous markings exactly as Reuter has described them. In other specimens, however, these colors are replaced by a testaceous, while the lighter markings of the typical form are obsolete and scarcely discernible.

Head longer than broad (25 : 21), eyes slightly shorter than width of vertex (9 : 10); antennal formula, 54 : 74 : 95 : 44; rostral formula, 36 : 27 : 13. Pronotum slightly shorter than

broad (male, 37 : 38; female, 42 : 45). Length, 8.5 to 10.5 millimeters; width, 1.6 to 1.8.

GORPIS FLAVICANS sp. nov. Fig. 1, d.

Pale flavous, shiny, thinly clothed with fine whitish pubescence; gula, sides of thorax, legs, and venter also with longer fine hairs; posterior lobe of pronotum coarsely punctate, hemelytra stippled with extremely fine, whitish punctures; head more or less entirely, eyes, ocelli, pronotum along the sides, first antennal segment beneath along basal half, a band on sides of anterior femora before the middle and another on apical third, and subapical ring on posterior femora faintly darker or rufescent; hemelytra somewhat whitish, the corium in greater part and the membrane translucent. Head longer than broad (27 : 23), vertex transversely depressed in front of ocelli; eyes prominent, the length of one slightly greater than width of vertex; ocelli about as far from each other as from the eyes. Antennæ slender, finely pubescent; formula, 53 : 74 : 90 : 42. Rostral formula, 30 : 27 : 13. Pronotum subequally as long as broad (male, 41 : 42; female, 47 : 48), the anterior lobe more gradually raised in front but more distinctly arched on the disc than in *sordidus*. Scutellum with raised apex broader and more truncate than in *sordidus*. Hemelytra extending beyond apex of abdomen; cells of corium (irregularly) and membrane translucent. Venter with a few longer hairs at apex. Male clasper with the blade produced at apex into a long sicklelike process (fig. 1, *b*).

Holotype, male, and allotype, female, Kolambugan, Mindanao, in collection of the United States National Museum. Paratypes, male and female, Basilan Island, in author's collection.

GORPIS PHILIPPINENSIS sp. nov. Fig 1, e.

Closely related to *G. flavicans* sp. nov.; size, proportions, and color similar, the females almost indistinguishable from those of that species, but the males easily recognized by the differently constructed genital claspers. The rufescent markings of anterior and posterior femora never (?) so distinct as they are in *flavicans;* the prothorax as measured from above slightly narrower across acetabulæ and across the postmedian pronotal constriction; anterior lobe of pronotum not so highly arched, its apex more gradually raised behind collar; and eyes slightly smaller and less prominent.

Clasper constructed on same plan but with outer portion of blade rectangular, the sides at apex produced into recurved

processes (fig. 1, *c*). Length, 9.5 to 11 millimeters; width, 1.7 to 2.1.

Holotype, male, Mount Maquiling, Luzon, and allotype, female, Penang Island, in collection of the United States National Museum. Paratypes, 3 males, Mount Banahao, Luzon, 1 male, Sibuyan Island, and 1 female, Penang Island, in collections of the United States National Museum and of the writer.

In 1909 Reuter [2] issued a monographic study of the genus *Gorpis* Stål, giving a diagnostic key to the seven known species. Unfortunately, the key was based, perhaps through necessity because of the absence of males, almost entirely on color characters. As is brought out by a study of the specimens before me, color, at least in some species of the genus, is so variable that feebly and strongly marked individuals of the same species will run to entirely different sections of Reuter's key. Furthermore, the females of many of the species, if one may judge the genus from the three forms before me, bid fair to be extremely difficult to separate structurally. It will not prove unlikely then, as our knowledge of the group becomes more nearly complete, that several of the names now in use shall fall into synonymy, since the greater portion of the species have been described from female examples. It is to be hoped that the males of more species will be discovered and the claspers, which are excellent specific characters, figured. The following is a catalogue of the species of the genus, with the known distribution, and with the sex and place of deposition of type specimens:

### Genus GORPIS Stål

Haplotype, *cribraticollis* Stål.

1. GORPIS ACUTISPINIS Reuter.

Mém. Soc. Ent. Belg. 15 (1908) 96.
Ann. Soc. Ent. Belg. 53 (1909) 425.
Madagascar. Type female, in Mus. Paris.

2. GORPIS ALBICANS Reuter.

Ann. Soc. Ent. Belg. 53 (1909) 426.
Insula Nias. Type female, in Mus. Genov.

3. GORPIS ANNULATUS Paiva.

Rec. Indian Museum 16 (1919) 370, pl. 36, fig. 4.
Assam. Type in Mus. Zool. Survey of India.

[2] Ann. Soc. Ent. Belg. 53: 423–430.

4. GORPIS APICALIS Reuter.

Ann. Soc. Ent. Belg. 53 (1909) 429.
Kilimanjaro, Africa. Type female, in Mus. Paris.
POPPIUS in Sjostedt's Kili.-Meru Exped. 12 (4) (1910) 59.

5. GORPIS CINCTICRUS Reuter.

Ann. Soc. Ent. Belg. 53 (1909) 428.
Madagascar. Type female, in Mus. Paris.

6. GORPIS CRIBRATICOLLIS Stål.

Öfv. Svenska Vet.-Akad. Förh. 16 (1859) 377.
Ceylon; Java. Type, female, in Mus. Berol.
DISTANT, Fauna Brit. Ind., Rhyn. 2 (1904) 398, fig. 254.
REUTER Ann. Soc. Ent. Belg. 53 (1909) 427.
POPPIUS, Tijd. v. Ent. 56 (1914) 181.

7. GORPIS ELEGANS Poppius.

Tijd. v. Ent. 56 (1914) 181.
Sumatra. Type in Mus. Helsingfors.

8. GORPIS FLAVICANS Harris, sp. nov.

Philippines. Types, male and female, in U. S. Nat. Mus.

9. GORPIS HUMERALIS (Distant).

*Dodonaeus humeralis* DISTANT, Fauna Brit. Ind., Rhyn. 2 (1904) 399, fig. 255.
*Gorpis* (*Dodonaeus*) *humeralis* REUTER, Mém. Soc. Ent. Belg. 15 (1908) 95.
Sikhim, India. Type, female, in British Mus.

10. GORPIS PHILIPPINENSIS Harris, sp. nov.

Philippines. Types, male and female, in U. S. Nat. Mus.

11. GORPIS RUFINERVIS Poppius.

Ann. Mus. Zool. Acad. Sci. 19 (1914) 138.
Victoria Nyanza, Africa. Type, female, in Mus. Petrop.

12. GORPIS SORDIDUS Reuter.

Ann. Soc. Ent. Belg. 53 (1909) 428.
New Guinea; Insula Deslacs. Types, male and female, in Mus. Hung.; Mus. Genov.

13. GORPIS SUBTILIS Reuter.

Ann. Soc. Ent. Belg. 53 (1909) 427.
Insula Fidschi. Type in Mus. Holm.

14. GORPIS TRANSVAALENSIS Schouteden.

Rev. Zool. Afr. 6 (1919) 241.
Transvaal. Type, female, in Schouteden collection.

# ILLUSTRATION

TEXT FIG. 1. Male claspers of various nabids; *a, Alloeorrhynchus bakeri* sp. nov.; *b, Nabis tagalicus* Stål; *c, Gorpis sordidus* Reuter; *d, G. flavicans* sp. nov.; *e, G. philippinensis* Stål.

# AN INQUIRY INTO THE SO-CALLED LATENT INFECTION IN YAWS-VACCINATED MONKEYS AS A POSSIBLE RESULT OF THE TEST FOR IMMUNITY BY INTRADERMAL INOCULATION WITH LIVING YAWS MATERIAL

By Isao Miyao[1]

*Of the Division of Biology and Serum Laboratory*
*Bureau of Science, Manila*

The theory that latent infection is the cause of resistance and not the consequence of immunity in treponematoses is still found in the literature. Therefore, one of the objections to the interpretation of the findings made in the test for immunity in yaws-vaccinated monkeys was the possibility that following the first infection, as a test for immunity, a latent infection developed in vaccinated and immune animals. However, the development of the resistance to inoculation in yaws-vaccinated monkeys was too sudden to be due to the infection as a test for immunity, and the resistance was found constantly in all vaccinated monkeys that were inoculated later than six weeks after the vaccination began.[2]

Nevertheless, it was deemed advisable to make an experimental inquiry into the possibility of latent infection in such animals, particularly in view of the great stress that is laid on this condition in experimental syphilis as an explanation of resistance to inoculation.

It was found by Schöbl and Hasselmann [3] that in yaws-infected monkeys the treponema of yaws may be detected in the regional lymph glands corresponding to the lesion while the lesion is still active, but not after the lesion has healed spontaneously. This condition, however, has not been investigated in monkeys immune as a consequence of yaws vaccination. I have, therefore, undertaken the following experiment.

[1] Lieutenant Surgeon, Imperial Japanese Navy.
[2] Philip. Journ. Sci. 42 (June, 1930).
[3] Philip. Journ. Sci. 35 (1928) 297.

From the series of monkeys vaccinated with killed yaws vaccine[4] and which were infected with live yaws material subsequent to the vaccination and found immune, all males were selected. Having been infected by intradermal inoculation with live material on the scrotum, these animals failed to develop yaws lesions and were particularly suitable for this experiment because the regional lymph gland corresponding to the place of inoculation could be easily located.

At intervals of time after the inoculation with living yaws material, as shown in Table 1, the inguinal glands corresponding to the place of inoculation were removed surgically and aseptically.

The glands were triturated in a sterile mortar and a small amount of physiologic salt solution was added.

The emulsion of the entire gland was taken up in a small hypodermic syringe and injected intradermally, following the method indicated by Schöbl.[5]

The small residue of the emulsion of the lymph gland was used for microscopic dark-field examination.

Treponemas in the skin lesions travel, or are carried, through lymphatic spaces and thus naturally reach the first barrier, the corresponding lymph gland, as pointed out by Schöbl.[6]

It becomes, therefore, important to know whether or not the treponemas reach the glands and remain there for a sufficient length of time following inoculation of the vaccine-immune monkey. Therefore, we proceeded to ascertain whether or not treponemas were present and alive in the regional inguinal glands of the yaws-vaccinated monkeys, following the test for immunity by intradermal injection of living yaws material, even though a skin lesion failed to develop.

For this purpose the following two procedures were applied:

1. Microscopic dark-field examination to search for treponemas in the glands.

2. Test of their presence and viability by means of inoculation into healthy animals.

In order to control the entire experimental procedure, inguinal lymph glands were removed from an animal with active

[4] Philip. Journ. Sci. 42 (June, 1930).
[5] Philip. Journ. Sci. 35 (1928).
[6] Philip. Journ. Sci. 35 (1928) 297.

scrotal yaws lesions, ground up, emulsified with physiologic salt solution, and inoculated intradermally into a healthy normal monkey.

The experimental animals consisted of five monkeys.

Four monkeys were vaccinated three times with killed yaws vaccine heated at 60° C. for one hour.

The interval between the time of lymphadenectomy and the test for immunity by intradermal inoculation with living treponemas is indicated in Table 1; namely, the first monkey three weeks three days, the second four weeks, the third six weeks, and the fourth eleven weeks after the last inoculation with live yaws material. The inguinal glands corresponding to the site of inoculation with yaws were removed and injected intradermally into normal healthy monkeys on the eyebrows (h–1, g–1, h–2, and g–2).

The inoculated animals were kept under observation for not less than two months; that is to say, twice the normal incubation period.

The microscopic examination of emulsified lymph glands by means of dark-field illumination resulted in negative findings, not only in yaws-immune animals, but also in the normal control animal with active yaws lesions.

From Table 1, it is evident that not a single yaws-vaccinated monkey harbored viable treponemas of yaws in the corresponding lymph glands.

The control nonimmune monkey that had an extensive active yaws lesion on the scrotum at the time the lymph glands were removed contained viable treponemas.

## CONCLUSIONS

Monkeys found immune to yaws when inoculated with live yaws material subsequent to yaws vaccination showed no evidence of so-called "latent infection."

## ACKNOWLEDGMENT

I wish to express my sincere thanks to Dr. Otto Schöbl, chief of the division of biology and serum laboratory, Bureau of Science, for guidance and assistance rendered in the course of this work.

TABLE 1.—*Showing the results of test for latent infection performed on yaws-vaccinated monkeys following inoculation with yaws.*

[+, typical yaws lesion; ±, atypical yaws lesion; —, no lesion.]

| Vaccine-immune donor monkey. | Last inoculation with yaws. | Result. | Lymphadenectomy. | Interval between last inoculation and lymphadenectomy. | Recipient monkey. | Result. | |
|---|---|---|---|---|---|---|---|
| | | | | | | Microscopic. | Take. |
| W–22–a | [a] VI– 4–28 | — | VI–28–28 | 3 weeks 3 days | h–1 | — | — |
| W–25–a | VI–26–28 | — | VII–24–28 | 4 weeks | g–1 | — | — |
| W–25–b | VI–25–28 | — | VIII– 9–28 | 6 weeks | h–3 | — | — |
| W–22–b | VI– 4–28 | — | VIII–20–28 | 11 weeks | g–2 | — | — |
| D–16. Control active yaws | V–24–28 | + | VII– 2–28 | 5 weeks 5 days | h–2 | — | ± |

[a] These letters and figures indicate month, day, and year: thus, VI–4–28 means June 4, 1928.

# IMMUNOLOGIC RELATION BETWEEN THREE PHILIPPINE STRAINS OF YAWS

By Isao Miyao[1]

*Of the Division of Biology and Serum Laboratory*
*Bureau of Science, Manila*

It has been proven experimentally by Schöbl that immunity with a homologous strain of yaws develops in Philippine monkeys within six months after inoculation, provided that a local yaw develops only at the place of first inoculation.[2] This finding has been confirmed by Tanabe.[3] It has been further demonstrated by Schöbl and Miyao that Philippine monkeys immune to yaws are also immune to cutaneous inoculation with syphilis.[4]

It was, therefore, to be expected that heterologous strains of *Treponema pertenue* would show likewise immunologic reciprocity. However, in view of the fact that some strains of yaws produce more-vigorous local lesions of longer duration than other strains in the same kind of animals, it was desirable to carry out experiments concerning the immunologic relation between several strains of yaws, particularly since Schöbl[5] has demonstrated that the strength and the rapidity of the onset of immunity in yaws depend on the extent and intensity of yaws infection.[6]

Three strains of yaws isolated in the Philippines and designated by the names of the patients were employed in this experiment. The animals were inoculated with one of the three strains and were superinoculated with another of the three

[1] Lieutenant Surgeon, Imperial Japanese Navy.
[2] Philip. Journ. Sci. 35 (1928).
[3] Philip. Journ. Sci. 40 (1929).
[4] Loc. cit.
[5] Philip. Journ. Sci. 35 (1928).
[6] Loc. cit.

strains at about the time when immunity to yaws sets in, according to the experiments with homologous strain of yaws performed by Schöbl.[7]

Of these three strains the one designated by the name "Kadangan" is a strain of yaws that was isolated from a patient in the Philippines by inoculation to Philippine monkeys, March 4, 1925. This strain has been thoroughly studied by Schöbl and kept alive to the present time by successive passages through Philippine monkeys.[8]

The second strain, designated "Guzon" strain, was also isolated from a patient in the Philippine Islands, November 15, 1928, and was passed through monkeys. It produced typical local yaw and distinct serologic reactions in the inoculated monkeys.

The third strain included in this experiment was the Katayama strain. It was isolated in January, 1928, from a Japanese who contracted yaws in Manila.[9] This strain was characteristic, producing in Philippine monkeys small and short-lived local lesions.

The tabulated results of our experiments show that immunologic reciprocity between heterologous strains of yaws exists. The time necessary for the development of immunity produced by infection with one strain of yaws towards another strain may vary somewhat; particularly if the immunizing strain produces feeble and short-lived lesions, the development of cross immunity may be delayed, as was the case with the Katayama strain.

ACKNOWLEDGMENT

Thanks are due to Dr. Otto Schöbl, chief of the division of biology and serum laboratory, Bureau of Science, for courtesies received in carrying out these experiments.

[7] Loc. cit.
[8] Loc. cit.
[9] Philip. Journ. Sci. 41 (1930) 13.

TABLE 1.—*Showing the results of cross immunity between heterologous Philippine strains of treponema frambœsiæ.*

[+, typical yaws; —, no yaws (immune); 0, not done.]

| Designation of monkey. | Inoculated with Kadangan strain of yaws. | | Superinoculated with Guzon strain of yaws. | |
|---|---|---|---|---|
| | Date. | Result. | Date. | Result. |
| B 6 | [a]IV–12–27 | + | XII–28–28 | — |
| Y–4 | V–31–27 | + | XII–28–28 | — |
| K–7 | I–15–27 | + | XII–28–28 | — |
| T 4 | VII– 2–26 | + | I– 8–29 | — |
| H 20 | XI– 8–26 | + | I– 8–29 | — |
| O–c | VII–27–26 | + | I– 8–29 | — |
| J–11 | III–19–26 | + | I–11–29 | — |
| D–8 | IV–25–25 | + | I–11–29 | — |
| Control | 0 | 0 | I– 8–29 | + |
| Do | 0 | 0 | XII– 28–28 | + |
| O–d | IV–11–27 | + | II–21–29 | — |
| T–15 | IX–17–27 | + | II–21–29 | — |
| W–6 | X–21–27 | + | II–21–29 | — |
| W–8 | X–25–27 | + | II–21–29 | — |
| W–13 | II– 7–28 | + | II–21–29 | — |
| W–36 | II– 1–28 | + | II–21–29 | — |
| W–43 | II– 6–28 | + | II–21–29 | — |
| W–45 | II– 7–28 | + | II–21–29 | — |
| W–49 | II–21–28 | + | II–21–29 | — |
| Control | 0 | 0 | II–21–29 | + |
| Do | 0 | 0 | II–21–29 | + |
| A–8 | VIII–17–28 | + | II–27–29 | — |
| o–1 | VI–21–28 | + | II–27–29 | — |
| E–41 | II–28–28 | + | II–27–29 | — |
| f–2 | VI–25–28 | + | II–27–29 | — |
| j–1 | VIII–17–28 | + | II–27–29 | — |
| j–18 | VI–26–28 | + | II–27–29 | — |
| K–9 | VI–26–28 | + | II–27–29 | — |
| W–51 | II–27–28 | + | II–27–29 | — |
| W–55 | V– 3–28 | + | II–27–29 | — |
| Control | 0 | 0 | II–27–29 | + |
| Do | 0 | 0 | II–27–29 | + |

[a] These letters and figures indicate month, day, and year: thus, IV–12–27 means April 12, 1927.

TABLE 2.—*Showing the results of cross immunity between heterologous Philippine strains of treponema frambœsiæ.*

[+, typical yaws; —, no yaws (immune); 0, not done.]

| Designation of monkey. | Infected with Guzon strain of yaws. | | Superinfected with Kadangan strain of yaws. | |
|---|---|---|---|---|
| | Date. | Result. | Date. | Result. |
| H–Guzon–24 | [a]I– 8–29 | + | VIII– 8–29 | — |
| | | | XI–21–29 | — |
| Guzon m–10 | II–21–29 | + | XI–19–29 | — |
| Guzon m–12 | II–27–29 | + | XI–19–29 | — |
| Control | 0 | 0 | VIII– 8–29 | + |
| Do | 0 | 0 | XI–19–29 | + |
| Do | 0 | 0 | XI–21–29 | + |

[a] These letters and figures indicate month, day, and year; thus, I–8–29 means January 8, 1929.

TABLE 3.—*Showing the results of cross immunity between heterologous Philippine strains of treponema frambœsiæ.*

[+, typical yaws; —, no yaws (immune); 0, not done.]

| Designation of monkey. | Infected with Katayama strain of yaws. | | Superinfected with Kadangan strain of yaws. | |
|---|---|---|---|---|
| | Date. | Result. | Date. | Result. |
| Katayama No. 1 | [a]I– 3–29 | + | VIII– 8–29 | + |
| | | | XI–21–29 | — |
| Katayama No. 2 | III–16–29 | + | XI–21–29 | — |
| Control | 0 | 0 | XI–21–29 | + |

[a] These letters and figures indicate month, day, and year; thus, I–3–29 means January 3, 1929.

NOTE: The terms treponema frambœsiæ and treponema luis are used for the sake of convenience and not as a suggestion for a new systematic nomenclature.

# IS FRAMBŒSIA TROPICA A NOSOLOGIC ENTITY?[1]

By Isao Miyao[2]

*Of the Division of Biology and Serum Laboratory*
*Bureau of Science, Manila*

## INTRODUCTION

The literature concerning the relation of yaws to syphilis is voluminous. A great many points of differentiation between the two diseases have been brought out. Not a few of these are irrelevant, while others are essential. A good many are of a purely speculative character. Others do not stand the scrutiny of critical experimenters. They fall into five realms of medicine; namely, parasitology, pathology and serology, clinical differential diagnostics and descriptive dermatology, epidemiology, and history of medicine.

The points of differentiation between lues and yaws are enumerated or tabulated in the literature, but only recently an attempt has been made(1) rationally to correlate the findings on which the differential diagnosis of frambœsia and syphilis rests. Thus, the student of tropical medicine was compelled to memorize every point, for fear that he might forget one or another of the differential diagnostic points which, for all the text books tell him, might be the very fundamentals involved in the question. His attention was centered upon the minute and doubtful tinctorial and morphologic differences between *Treponema pallidum* and *Treponema pertenue*, then he was transferred centuries back and confronted by Christopher Columbus, who upon his return to Europe, as some theories would have it, brought with him frambœsia; that is, tropical syphilis.

The long line of arguments, arrayed in favor of the dualistic nature of yaws and syphilis, it would appear, carries insufficient weight to silence the minority who argue that these two diseases are the same. By emphasis on epidemiologic

[1] Presented as thesis for the degree of "Hakushi" in Medicine and prepared under the direction of Dr. Otto Schöbl, chief of the division of biology and serum laboratory, Bureau of Science, Manila.

[2] Lieutenant Surgeon, Imperial Japanese Navy.

observations and on those particular phases of clinical manifestations of one disease, which by themselves cannot be distinguished clinically from analogous lesions of the other disease, the fundamental biologic differences in the nature of the two infections are pushed into the background, and a wide space opens for speculation. Misconception of immunity in general, and of the brand of immunity in particular that is encountered in syphilis, yaws, vaccinia, variola, fixed and street virus of rabies, and perhaps a few more, as well as wrong interpretations of immunologic findings in yaws and syphilis, helped to envelop the problem of identity or duality of these two diseases in a fog, almost mystical, that leads some to seek the solution of the problem in the fifteenth century rather than in present and future experimental and laboratory investigations into the fundamental biologic characteristics of the treponema that causes the disease clinically known as syphilis, and of the treponema that causes the disease clinically known as frambœsia tropica.

In the face of the occasional difficulties in differential clinical diagnosis and the impossibility, for the present, of the differentiation—by morphological, tinctorial, and cultural methods—of treponema luis from treponema frambœsiæ, speculative arguments based on epidemiology and the history of medicine contribute to practical medicine but a sense of confusion and uncertainty. Furthermore, they have no place in concrete science, such as medical research, because they offer at present no tangible proof.

## I. AN INTERPRETATION OF THE DIFFERENCES BETWEEN FRAMBŒSIA AND SYPHILIS IN THEIR CLINICAL MANIFESTATIONS AND THEIR DIFFERENT BEHAVIOR WITH REGARD TO HEREDITY, GEOGRAPHIC DISTRIBUTION, INCIDENCE AS TO AGES, TRANSMISSION, IMMUNITY, AND CHEMOTHERAPY.

In the following pages an attempt is presented at an interpretation of the amassed arguments relating to the points of differentiation between the two diseases. As a basis for this interpretation distinct biologic differences between treponema luis and treponema frambœsiæ were considered. It will be seen that without any stretch of the imagination the various observations, offered as points of differentiation between syphilis and yaws, can be rationally correlated with the fundamental biologic peculiarities of the causative agents, as far as known to-day. This correlation is achieved by the application of well-known

and substantiated general principles that have been adopted in basic medical sciences. The entire mass of evidence under discussion can be thus logically reduced to one or two problems. Thus the fundamentals become more evident, and the direction is indicated in which the principles underlying the peculiar biologic differences are to be sought. Those phases of syphilis and yaws that form the clinical border line between the two must be kept out of consideration while the framework of the double structure is being erected, so that we may see whether the double structure will withstand the weight of criticism or whether one of them will collapse.

The early hope of morphologic or tinctorial differentiation of "*Spirochæta pallida*" from "*Spirochæta pallidula seu pertenuis*" has not been realized. The question of the cultural differentiation of the two treponemas still awaits answer. Beyond their successful cultivation no further progress has been made. The once-claimed qualitative distinctions in serology of syphilis and yaws have not been substantiated in later days.

Thus we are restricted for the present largely to a comparison of biologic properties, as revealed by the behavior of treponemas in the body organism and by the tissue response to the infection. However, this need not discourage anyone. The vaccine virus became well known in its biologic properties and the knowledge was fully taken advantage of by practical medicine without the virus itself having ever been seen or cultivated.

The fundamental biologic difference, as far as known at present, between treponema luis and treponema frambœsiæ lies in the pathogenesis of syphilis and yaws.

### BRIEFLY DEFINED

The treponema frambœsiæ is epiblastotropic. The treponema luis is panblastotropic with preference to mesoblastic tissues. This definition becomes clear in the course of further discussion.

### 1. INITIAL LESION

| YAWS | SYPHILIS |
|---|---|
| Treponemas invade epidermis and multiply consequently. | Treponemas invade epidermis and multiply consequently. |
| The primary efflorescence of the initial lesion is a papule. | The primary efflorescence of the initial lesion is a papule. |
| Treponemas remain in the superficial layer of the skin; | Treponemas penetrate early into deeper layers of the skin; |

consequently, soft œdema or none collects. The œdematous liquid oozes out.

Treponema frambœsiæ localizes in epidermis; consequently, the reaction on the part of the affected tissues is downgrowth of epidermis and upgrowth of granulation tissues. The resulting efflorescence is a papilloma—a yaw. It produces no early change in the blood vessels nor deep œdema; the result is profuse oozing and absence of early ulceration.

Treponemas remain localized in the superficial layers of the skin; consequently, the healing of the early yaws is either by restitution ad integrum or by flat thin superficial scar.

Treponemas show preference to skin; consequently, the initial lesion in a great number of cases persists to and beyond the early metastatic skin eruptions, which in turn last long. The primary lesion lasts so long that it frequently changes into an ulcer of late, so-called tertiary, character.

consequently, deep hard œdema collects at the basis of the lesion. No oozing sclerosis.

Treponema luis penetrates early into the deeper layers of the skin and produces early changes in blood vessels and deep œdema; consequently, the reaction on the part of the affected tissues is necrosis surrounded by deep hard œdema—a chancre.

Treponemas penetrate early into the deeper layers of the skin; consequently, the healing of a chancre is accomplished by a more or less deep branching cicatrization.

Treponemas show mesoblastic preference and penetrate deeply and rapidly into the skin and internal organs; consequently, the chancre heals before the advance of early metastatic eruptions, which in turn are also of short duration.

A chancre heals early and never changes into an ulcerative lesion of late, so-called tertiary, character (gumma).

### 2. LOCALIZATION OF INITIAL LESION

#### YAWS

Treponemas are epiblastotropic; consequently, the initial lesion localizes on the skin on the mucocutaneous border of the nose and the mouth, and

#### SYPHILIS

Treponemas are panblastotropic; consequently, initial and early metastatic lesions localize on the skin, on the lip, on the conjunctiva, on the ex-

on the mucocutaneous border of the external genitals (high altitudes) (2) and the anus.

ternal genitals, at the introitus ad vaginam, on the cervix uteri in the urethra, on the tongue, on the palate, and on the mucous membranes of the rectum.

### 3. IMMUNITY

| YAWS | SYPHILIS |
|---|---|
| Treponemas are localized superficially in the early lesions and do not produce early vascular changes, do not penetrate into the deep layers of the skin, invade the lymphatic system and the blood stream only temporarily and do not persist there, and do not localize permanently in other organs; consequently, the immunity is late in developing. | Treponemas penetrate early and establish themselves in the deep layers of the skin, in the lymphatic system, and through the blood stream in the internal organs and produce early vascular changes. Immunity develops much earlier. |
| Immunity persists after the treponemas disappear from the tissues. | The so-called latent infection persists coincident with immunity. |
| (Immunity without concomitant, so-called latent, infection.) (3, 4) | |
| Treponemas of yaws immunize against themselves sooner than against treponemas of syphilis. (1) | Treponemas of syphilis immunize against themselves far sooner than against treponemas of yaws. |

### 4. METASTATIC LESIONS

| YAWS | SYPHILIS |
|---|---|
| Due to late development of immunity the metastatic lesion is identical in character with the initial local lesion. Modified metastatic lesions develop later at the time when immunity begins to develop. Therefore, early metastatic lesions are more uniform. | Due to early onset of immunity the metastatic lesions are different in character from the initial local lesion; therefore, greater variety of metastatic skin manifestations. Metastatic lesions do not resemble the initial lesion—that is, the chancre. |

Treponemas are epiblastotropic and do not colonize internal organs; therefore, when cutaneous manifestations disappear and the skin is completely sterilized, no relapses occur.(3)

Treponemas are panblastotropic and colonize internal organs; consequently, with healing of skin manifestations the process is not finished, but relapses occur, caused by invasion of tissues by treponemas stored in the internal organs.

### 5. CHEMOTHERAPHY

YAWS

Due to exclusive localization of treponemas in the skin and not in other tissues, frambœsia in the early stage is exquisitely amenable to chemotherapeutic treatment.

SYPHILIS

Treponemas are localized in the skin and other tissues, and skin manifestations of syphilis are promptly amenable to chemotherapeutic treatment, but infection continues from mesoblastic sources.

### 6. TRANSMISSION

YAWS

Treponemas are epiblastotropic, and lesions are confined to skin transmission by direct contact and by insects feeding on the discharge of efflorescences.(5)

Treponemas do not localize permanently in lymphatics and blood vessels nor in internal organs;(6) therefore, not in placenta and congenital transmission is absent.

SYPHILIS

Treponemas are panblastotropic, and initial and metastatic lesions are localized around and in body orifices. Transmission is by direct contact and through sexual intercourse (venereal disease).

Treponemas are panblastotropic and localize in lymphatics, blood vessels, internal organs, and placenta permanently; therefore, congenital transmission occurs.

### 7. INCIDENCE AS TO AGES AND LOCALITIES

YAWS

Transmission of epiblastotropic treponemas by direct contact of skin and by insects is responsible for heavy incidence among children and in rural districts.

SYPHILIS

Venereal disease is responsible for heavy incidence after puberty and in large cities.

Treponemas are highly susceptible to low temperature,(5) epiblastotropic; therefore, yaws is influenced in clinical manifestations and geographic distribution by climate; a tropical disease.

Primary and metastatic lesion is specially localized on warm and moist surfaces and body orifices, sexual transmission; therefore, not influenced by climate. Moreover, the treponema of syphilis is far more resistant to adverse conditions prevailing outside of body than the treponema of yaws;(7) therefore, pandemic.

## II. DISCUSSION OF THE INTERPRETATION

The differences between yaws and syphilis in their pathogenesis as just pointed out may at first sight look to be more a matter of degree than of kind. However, there are observations of two phenomena which constantly permeate the histopathologic changes and are constantly met with in syphilis and are not found in frambœsia. They are:

1. The localization of treponemas in the epidermis and the lack of vascular changes in early yaws.
2. The localization of treponemas in the corium and mesoblastic tissues and the pronounced vascular changes in syphilis.

These two facts are well known and should not require any further comment. Nevertheless, I am reproducing here the results of histopathologic comparative study of typical yaw (papilloma) and an early skin manifestation of syphilis which resembles a yaw more than any other syphilide, that is condyloma. For the sake of fairness I prefer to use findings of others rather than my own.

The following was abstracted from Hallenberger:(8)

### 1. HISTOPATHOLOGY OF YAWS AND SYPHILIS

| YAWS (PAPILLOMA) | SYPHILIS (CONDYLOMA) |
|---|---|
| The lesion is covered with epidermis. | The lesion is covered with epidermis. |
| Corneal and granular layers are gone; in their place, a lamellar layer of flat nucleated cells is found. | Corneal and granular layers are gone; in their place, a lamellar layer of flat nucleated cells is found. |

The spaces of these layers are filled with serum, red blood cells, leucocytes, and detritus.

Rete Malphigi shows enormous downgrowth reaching clear to the subcutis.

The suprapapillary epithelial layer is moderately thickened.

The interspinal spaces of the epidermis are dilated and filled with polymorphonuclears. They lead frequently to the formation of miliary abscesses located within the epidermis.

The basal layer of the epidermis shows extensive leucocytic infiltration which extends to papillary bodies. The leucocytes are so numerous that the epithelial cells are subdued and the dividing line between the papillæ and the epidermis is indistinguishable.

The papillary and subpapillary layer of the corium shows loose cellular infiltration which consists in the centrum of plasma cells almost exclusively, while towards the periphery mononuclears and fibroblasts are also present. Towards the surface (epidermis) polymorphonuclears are predominant. This loose infiltration follows the branching blood vessels like sheets surrounding blood vessels and glands. Giant cells not found. Blood vessels distended show no changes in their walls.

Levaditi stain shows that treponemas are present only in

The spaces of these layers are filled with serum, red blood cells, leucocytes, and detritus.

Rete Malphigi shows slight downgrowth.

The suprapapillary epithelial layer is slightly thickened.

The interspinal spaces of the epidermis are dilated and filled with polymorphonuclears. They lead frequently to the formation of miliary abscesses located within the epidermis.

The papillary and subpapillary layer of the corium shows thick, sharply outlined, cellular infiltrations, which follow the blood vessels. They are composed of round cells, plasma cells, fibroblasts, and giant cells. These infiltrations are found in the deep layers of the corium and sometimes even in the subcutis arranged along the blood vessels. The walls of the blood vessels show cellular infiltration and their endothelium is hypertrophic to such a degree as to cause almost complete obliteration.

Levaditi stain shows that treponemas are predominant

| | |
|---|---|
| the epidermis within the polymorphonuclear infiltrations. | in the corium and the papillary layer. They are also found within the walls of the blood vessels. |

In view of the general knowledge of these two biologic differences between yaws and syphilitic infection, as just recapitulated in the quotation from Hallenberger, these two fundamental biologic differences between yaws and syphilis must be accepted as valid. The deductions, however, I have made in an attempt to explain the differences between yaws and syphilis as given in literature, need further discussion. We shall now discuss the individual points, one by one.

The initial lesion in yaws is a papilloma,(8) in syphilis it is a chancre. Taking the fact that treponema frambœsiæ remains localized in epidermis as established, we can follow the development of a papilloma histologically. Even though at times treponema frambœsiæ is found in a rather deep layer of the lesion, it will be confined to those portions of the lesion that are composed of epidermal columns that have grown deep down into the skin, at times reaching the subcutis. The downgrowth of the epidermal cells in a form of deep columns (acanthosis) must be considered as a reaction on the part of the epidermis to the invasion of the treponema frambœsiæ. The cutis proper responds at a distance to the epidermal invasion by sending leucocytes, predominantly polymorphonuclears, into the epidermis and by upgrowth of the inflammatory granulation tissue of the corium. Blood vessels, however, are not affected. The irritation on the part of the infection is such that the corium responds predominantly by plasmatic cell reaction. The superficial localization in the skin of the yaws lesion naturally is accompanied by soft œdema that is not very much pronounced, because the removal of the lymph from the pathologic tissue by oozing from the large surface is facilitated. There is no change in the blood vessels that would lead to the narrowing or obliteration of the lumen and consequently to necrosis.

In syphilis, on the other hand, the treponema luis penetrates early and is present in the largest numbers in the corium; consequently, the reaction on the part of the corium is more pronounced than on the part of the epidermis. Therefore, the exudate in this early period is predominantly composed of lymphocytes rather than polymorphonuclears. The downgrowth

of the epidermis is not so pronounced and the lesion being located in the corium, the œdema is deep, oozing is not pronounced, and therefore the liquid part of the exudate accumulates within the tissues of the cutis and even subcutis which become indurated. The early changes in the blood vessels lead to the narrowing of the lumen of the blood vessels and even to complete obliteration, which phenomenon combined with the pressure of the interstitial œdema of the cutis is generally taken in pathology as the cause of necrosis; consequently, the primary lesion is a sclerosis and a defect of the superficial part of the skin surrounded, and sets on a deep indurated base.

Yaws heal by restitution or superficial scar formation; syphilis by more or less deep branching scar.

What has been said about the differences in the primary lesion of yaws and syphilis is sufficient explanation for the differences in the results found in the healing of yaws and syphilis. It stands to reason that a superficial predominantly epidermal lesion will more likely heal by restitution or superficial scar than a deep pathologic skin lesion accompanied by necrosis.

The initial lesion in yaws is apt to last much longer than that in syphilis.

The explanation we offer is, of course, deduction from clinical and experimental observations correlated with the fundamental biologic differences of the two treponemas. Relatively little is known about the mechanism of the healing of syphilitic or of yaws lesion. The conception as expressed in the claim(9) that the healing is independent from the development of resistance to superinoculation or reinoculation is founded on sufficient experimental evidence both in humans and in experimental animals. However, little is known about the mechanism of healing. That healing is coincident with destruction of treponemas in the yaws or syphilitic lesion there is no doubt. Following a chemotherapeutic dose the treponemas disappear from the lesion within a few hours; and the early skin lesions, particularly in yaws, melt away, so to speak, before our eyes as a consequence thereof. This proves that with the disappearance of the treponemas from the lesion the lesion heals. This is an established fact observed by many investigators. About the mechanism of the spontaneous healing of treponematous skin lesion we are still in the dark. There are some few points, however, that have been established, and others that indicate the possibility of solving even this problem by experimental investigation.

No indication was found of humoral curative immunity in yaws. Repeatedly, no effect was achieved upon the healing of yaws lesion by injecting directly into the lesions the serum collected from highly resistant animals or man. Another observation, however, seems to point to at least one factor that may be responsible, under natural conditions and without the aid of chemotherapeutic treatment, for the removal and destruction of the treponemas and consequent healing provided they are prevented from multiplying by immunity. It was noticed that whenever intradermal inoculation of yaws material was followed by acute inflammation with pus formation, due to admixture with the pyogenic microbes, the development of a local yaw at the place of inoculation was either greatly delayed or a yaw did not develop at all. This apparently insignificant observation seems to gain weight in connection with the observation of Goodpasture(12) on healing yaws subsequent to theraphy and in connection with studies of Bergel(13) on the influence of leucocytes and their extract upon the changes in morphology and tinctorial behavior of treponema luis. Goodpasture noticed that an extensive disintegration of lymphatic elements and leucocytes takes place within the yaws lesion during the process of healing after injection of neosalvarsan. Bergel made extensive studies on the behavior and changes of *Treponema pallidum* in an animal's body, particularly in the peritoneal cavity, in which he convinced himself of what appeared to be a direct effect and destruction of *Treponema pallidum* by leucocytes, lymphatic elements, and even extracts from lymphatic organs. The complete destruction was preceded by gradual deterioration of *Treponema pallidum.* This process could be delayed or prevented by a colloidal lipoid, such as lecithin. The problem of healing of the treponematous lesion is far from approaching solution, but the importance of lymphatic and leucocytic elements is constantly coming into view.

### 2. LOCALIZATION OF TREPONEMA FRAMBŒSIÆ AND OF TREPONEMA LUIS

Treponema frambœsiæ is localized superficially and does not produce early vascular lesions; consequently, immunity develops late.

Treponema luis penetrates early into the mesodermic tissues and produces early changes on the vascular system; therefore, immunity develops early.

There is a sufficient amount of recent experimental evidence on immunity to yaws in man and in experimental animals, in which the infection runs a very similar course to that in man, that the immunity to yaws does not develop until after about eight months in man and after six or seven months in the Philippine monkey with local yaw. If we compare these findings with experimental findings on man and monkeys in experimental syphilis we will see that the immunity to syphilis in man starts to develop in fifteen days and is completely developed in thirty days, while in monkeys it develops in forty-five days. This difference in behavior of the two infections with regard to immunity can be logically correlated when the results of subcutaneous immunization of monkeys to yaws is considered.(14) While monkeys that were infected intradermally with yaws were reinoculable for five months and some for even six months, when the yaws material was injected subcutaneously without development of yaws the immunity set in earlier than four months after the initial immunization. In my opinion it seems to indicate strongly that if the treponema frambœsiæ was not barred from penetration into the mesodermic tissues at an early stage of the infection, the immunity would develop much earlier than it does under the natural or experimental conditions where it is placed and maintains itself in the epidermis. That the penetration into the deeper tissues of the treponema frambœsiæ has an influence upon the development of immunity is further evident from the repeated observations on experimental monkeys that developed generalized yaws. If the generalized yaws developed early—the earliest observed took place in an experimental monkey in the third month—the immunity sets in early, that is about the fourth month instead of the seventh. In every case of generalized experimental yaws the immunity develops much earlier than it does as a consequence of local yaw. A similar observation was made on experimentally infected humans. It was noticed that yaws patients became immune to superinfection during the stage of generalized yaws, while patients that exhibited late yaws manifestations were reinoculable for a much longer period of time.(15)

### 3. THE SKIN LESIONS IN YAWS ARE MORE UNIFORM THAN THOSE IN SYPHILIS

Due to late development of immunity in yaws, metastatic lesions are identical with the initial local lesion; and modified metastatic lesions develop at the time when resistance to super-

infection has partly developed. Therefore, the early skin lesions in yaws are more uniform.

The period of incubation of generalized, so-called secondary, yaws in humans and in monkeys was found by experiments to be the same; that is, ten to twelve weeks. However, the development of immunity to yaws in humans and to local yaw in monkeys was found to be about six months. Consequently, there are three full months during which the yaws manifestations appear, lesions cropping out on the skin without any interference whatsoever on the part of the immunity. The metastatic lesions, therefore, appear to be of the same general character as the primary lesion that is a yaw. It was observed on human volunteers and on experimental monkeys that the protean lesions developed towards the end of the generalized process, being either admixed to typical or almost typical yaws or cropping out without a typical yaw developing at the same time. The previous metastatic typical yaws, of course, may still persist, but in a given case—which may be earlier in one case than in another—the eruptions of the protean lesions are noticed as the last number on the repertory of the generalized yaws skin manifestation. In syphilis on the other hand the early metastatic, so-called secondary, lesion never resembles a fully-developed primary lesion. The closest that a metastatic, or secondary, syphilis in man ever resembles the primary syphilitic lesion is the elevated and rather broad papule covered with a crust, the papule-caustous syphilide.

Finger(16) describes the course of experimental syphilis in monkeys as follows:

> The small wounds caused by the inoculation first heal. But then after an incubation of ten to forty-two on the average twenty-one days there forms at the place of inoculation small spots of reddening, the center of which soon becomes elevated in a form of a nodule. These nodules grow to the size of a lentile and become covered by crust due to superficial disintegration. Under this crust flat sharply outlined reddish yellow erosions are found which exude sanguinolent yellowish serum. By spreading and by confluence of the neighboring erosions more extensive map-like ulceration results which sometimes covers larger areas, for instance almost the entire lid. After existing for variable time but always for several weeks the ulceration heals and leaves either pale or (in case of cynokephalo and corcopithecus) scars inclosed by pigmented margin. The base of the ulceration shows sometimes slight infiltration. The base of the ulcer is somewhat elevated but not particularly indurated while in Anthropoid apes the induration and therefore the resemblance to sclerosis is pronounced. In lower monkeys the course of syphilis terminates mostly with the healing of the primary lesion. In relatively rare cases there appear several weeks after the healing of the initial lesion narrow semicircular infiltrations surrounding the scar. These infiltrations spread towards the periphery in the

form of serpignimous desquamative papules. At times they reach considerable extent and heal leaving pigmentation. In case of the Anthropoid monkeys particularly in chimpanzee there develop, about eight to ten weeks after the inoculation, manifestations of generalized secondary syphilis. Maculous, papulocrustous exanthema on the general integument, the palms of the hands and soles of the feet, ulcerated papules on the mucous membranes.[3]

The immunity in man with regard to treponema luis, as quoted from Neisser,(17) sets in at the end of the first month and is seen to develop at the end of the first two weeks of the duration of the primary lesion.

Treponema frambœsiæ is epiblastotropic and does not gain a foothold on mucous membrane. Treponema luis is panblastotropic and gains a foothold on mucous membrane.

Neither primary nor secondary lesions of yaws occur on mucous membranes. In syphilis, on the contrary, it is a place of predilection. The localization of treponema frambœsiæ in the epidermis and its inability to maintain itself in the mesoblastic tissues is the reason for this different behavior of syphilis and yaws. That absence of primary lesion on the mucous membrane is not accidental, and is a fundamental difference, is brought out by the fact that metastatic yaws lesions likewise do not occur on mucous membrane. The only condition under which a yaws lesion localizes on mucous membrane is by spreading from the mucocutaneous border onto the mucous membrane.(18) This is made possible by the formation of a crust. The oozing and the exudation at the base of the crust make it possible for the treponema frambœsiæ secondarily to maintain itself in a lesion located on the mucous membrane. We see at once the different reaction on the part of the mucous membrane from that of the epidermis. There is no downgrowth of the epithelial cells of the mucous membrane proper such as we see in a skin lesion and the lesion propagates itself by crust containing oozing lymph, disintegrated leucocytes, and superficial epithelial cells of the mucous membrane. In this early lesion it lies adjacent to the membrana propia, is long preserved, and shows no proliferation and penetration in the deep layers of the mucous membrane proper. The reaction on the part of the mucous membrane proper is similar to that encountered in the corium; that is, cellular and œdematous infiltration and plasma-cell accumulation.

[3] Quoted from W. Kolle und H. Hetsch, Die experimentelle Bakteriologie und die Infektionskrakheiten, sechste Auflage, zweiter Band, 873.

Conditions are different in syphilis. The treponemas, having penetrated into the mucous membrane through the slight epithelial erosion, are able to gain a foothold and to propagate in the mucous membrane proper. Therefore, syphilis in its initial and metastatic lesion is manifested on mucous membrane.

It is generally recognized in the literature concerning yaws that neither primary nor metastatic yaws lesions locate on the mucous membrane. The reason for this deviation from the way of localization of syphilis must be sought in the biologic difference; that is, the predilection of treponema frambœsiæ for the epidermis and the predilection of treponema luis for the mesoblastic tissues. The reason for this difference, of course, is difficult to understand, particularly in view of the fact that, as has been frequently observed, both by clinicians and experimenters, an original skin lesion will spread on the mucocutaneous border and from there on to the mucous membrane. Whether or not the gradual transition of epidermis into the mucous membrane with stratified epithelium makes this possible it is difficult to say at present. However, in the absence of the general reason for tissue tropism we cannot give even a suggestion of the underlying principles that are responsible for the epiblastotropism of treponema frambœsiæ and the panblastotropism and mesoblastic tissue preference of treponema luis. However, careful and persistent observation of the development of lesions in experimental investigation on man and susceptible animals brings out the fact that treponema frambœsiæ does not localize on mucous membrane either as a primary lesion or as a metastatic lesion. We may speculate as to the reason for it and say that either the epidermis contains a substance which is necessary for the growth of treponema frambœsiæ, a substance which is absent in other tissues; or, what may be considered more likely, we may say that the epidermis is so differentiated and so void of other tissues that the treponemas are without the reach of the natural defenses of the body. Mucous membranes have usually a very thin epithelium and lie immediately on a layer composed of mesoblastic tissues. We have seen, however, sections prepared from a frambœsia lesion that spread over the mucous membrane covered with high epithelium. It was observed in these sections that the frambœsic process in such a mucous membrane spread on the epithelium alone, while a relatively small reaction at first is provoked in the mucosa proper. Syphilis, on the other hand, is panblasto-

tropic; and the treponemas luis, as soon as they make their way through the integument of skin or mucous membrane, penetrate rapidly into the depth of the skin or the mucous membrane and find themselves at once in a tissue which they prefer. Consequently, both primary and metastatic lesions on mucous membranes occur in syphilis that have no connection whatsoever with the lesions on the skin, either past or present.

### 4. YAWS IS NOT CONGENITAL; SYPHILIS IS CONGENITAL

From what has been said as to the localization of treponema frambœsiæ and consequently of the localization of the primary and metastatic lesion it need not to be mentioned again that the inability of treponema frambœsiæ permanently to localize and produce lesions in internal organs and, in particular, its inability to attack blood vessels is responsible for the absence of hereditary transmission in yaws. Syphilis, on the other hand, commonly attacks the blood vessels and prefers mesoblastic tissues. Foci of treponema luis are deposited in internal organs, including the blood vessels, and syphilitic anatomical changes in the placenta are common. Therefore, the congenital transmission of syphilis is obvious.

## CONCLUSIONS

Biologic differences in organotropism or tissue selectivity between the treponema of yaws and the treponema of syphilis are responsible for the difference between these two diseases with regard to their clinical course, severity of symptoms, heredity, and epidemiology.

The two diseases belong to one group and show close relationship, but are fundamentally distinct.

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NOTE: The terms treponema frambœsiæ and treponema luis are used for the sake of convenience and not as a suggestion for a new systematic nomenclature.

# A SURVEY OF PROTOZOA PARASITIC IN PLANTS AND ANIMALS OF THE PHILIPPINE ISLANDS [1]

By Robert Hegner

*Professor of Protozoölogy, Johns Hopkins University, Baltimore*

and

H. J. Chu

*Instructor in Zoölogy, Ginling College, Nanking*

FIVE PLATES

## INTRODUCTION

So far as known to the writers only a few records have been published of parasitic Protozoa from animals of the Philippine Islands and none from the plants of this region. A knowledge of this subject is of scientific and practical importance. Parasitic protozoans are responsible for many diseases in both domestic and wild animals as well as in man, and certain species occur in plants and may be pathogenic to them. Parasitology has become one of the major subjects in schools of medicine and of hygiene and is of particular interest in the Tropics since parasites are more numerous and seem to be more pathogenic where tropical conditions exist than in temperate regions. An effort has been made by us to examine for protozoans a large number of domestic animals and the commoner wild animals and plants that may be obtained easily for study. The results here published are fragmentary and serve only as the beginning of a large project. They should be of value to students and teachers of parasitology.

Protozoa living in man are not always available but those of lower animals can usually be secured without difficulty; many of these belong to genera that occur in man and several, ap-

[1] From the Department of Parasitology, School of Hygiene and Public Health, University of the Philippines. We wish to express our appreciation of the facilities placed at our disposal by the Director of the School of Hygiene and Public Health and of the assistance of our colleagues, especially that of the Secretary, Dr. Hilario Lara, and of our assistant, Mr. F. S. Manipol.

parently, are of the same species. The life cycles and the methods of transmission of the Protozoa of lower animals are similar to those of the human species, and we can often determine the activities of the latter by observations on species from lower animals. For example, the process of fertilization in the life cycle of the human malarial parasites was first observed by MacCallum (1897) after he had followed the process in the *Haemoproteus* of birds; this discovery played an important rôle in the working out of the mosquito transmission of malaria by Ross (1898), which was also first accomplished with parasites of birds and later was found to be similar to that of human malarial parasites. More recently a new drug, called plasmochin, has been developed as a result of experiments with birds that is effective against human malaria and will probably prove to be a valuable addition to the methods of controlling this disease in man. The Protozoa of lower animals also afford material for experimental purposes. Human beings may sometimes be employed as experimental animals, as in the work of Walker and Sellards (1913) on amœbæ, which was carried out in Manila and is the best experimental work ever done on this subject, but only under exceptional circumstances can such studies be made. Biological surveys of parasitic Protozoa are of interest as they often reveal species previously unknown, add to our knowledge of the distribution of the Protozoa, and furnish evidence of value in studies of the relationships of hosts.

We have studied the parasitic Protozoa of monkeys more thoroughly than those of any other type of lower animal because most of the Protozoa that have been reported from monkeys seem to be morphologically indistinguishable from those living in man. The details of these studies on monkeys will be published in a separate paper. The other animals studied were selected on the basis of availability. They are, for the most part, animals that are easily obtainable in the vicinity of Manila. The brief statements regarding the structure, the life cycles, and the distribution of the types of parasitic Protozoa described, as well as the drawings, have been included as an aid to the discovery and identification of these organisms.

Surveys of the fauna of any region are usually devoted at first to the larger types of animals, such as mammals, birds, reptiles, fishes, etc., or to the more conspicuous species, such as butterflies and dragon flies. Only after these and other groups have been studied do the smaller animals attract attention, and

usually among the last of these to undergo investigation are the parasites, especially protozoan parasites. We are accustomed to think of the naturalists who risked their lives to collect species of Philippine birds and mammals during the nineteenth century as pioneers, and to believe that work of that type is no longer possible, but a protozoölogist who, at the present time, undertakes a survey of either the free-living or parasitic Protozoa of the Philippine Islands is essentially a pioneer, since practically no work on this subject has been done. Certain species of medical importance have been investigated with considerable care, especially the malarial parasites, the dysentery amœba, *Endamœba histolytica,* and the dysentery ciliate, *Balantidium coli,* but no doubt thousands of species, many of which are new to science, live on or within the bodies of Philippine animals. That this is true is indicated by our results presented in this paper. We have not been able to study in detail any of the organisms recorded except those that live in monkeys and the *Giardia* of the civet cat, which will be described in separate papers. It has thus been impossible for us to determine in most cases the specific identity of the Protozoa found, but we hope that our studies may stimulate further investigations.

## PROTOZOA OF MAN

Although our observations have been devoted almost entirely to the Protozoa of the lower animals, we have made a few observations on the Protozoa of human beings. During a visit to Olongapo, Zambales, we obtained fresh fæcal samples from thirteen Negritos and examined them at once.[2] Slides were also prepared for future study. We found that every one of the thirteen was infected with hookworms and amœbæ and that four of them were infected with *Trichomonas hominis. Endamœba coli* was present in every individual and *Endolimax nana* in seven of them.

No adequate survey has been made of the parasites of the non-Christian tribes living in the Philippine Islands, and more careful studies of Christian Filipinos are very desirable. At least one hundred individuals of each type should be taken as a sample, and fæcal and blood examinations for Protozoa and worms should be made. The results should be considered in

[2] We are indebted to Mr. de Aro and Mrs. John Gordon, of Olongapo, for making it possible for us to obtain this material.

connection with the character of the food and habits of the people that might have an influence on the transmission and reproduction of the parasites.

## PROTOZOA OF MONKEYS

For purposes of classroom material and of research the Protozoa of monkeys are peculiarly valuable. The studies of many investigators have revealed that morphologically the Protozoa that live in monkeys are indistinguishable from those that live in man. They are also similarly located. An autopsy on a monkey affords even better material for study than an autopsy on a human being since monkeys are more frequently infected with a large number of species than are human beings. This, at least, is true of the monkeys that we have examined from Luzon. A suggested plan of procedure is first to obtain samples of blood from the living monkey; this can most easily be accomplished by snipping off the end of the tail. A drop of living blood should be spread out under a cover glass and examined at once to detect trypanosomes or any other active, blood-inhabiting Protozoa, and several films should be made and stained with Giemsa for later examination for malarial parasites and babesias, both of which have been reported from monkeys. A sample of fresh blood is particularly valuable since an active trypanosome can easily be found, whereas if only one or two trypanosomes are present on a stained film they can be located only after tedious search. Blood films in the Tropics should be dried and fixed with absolute methyl alcohol as soon as possible in order to prevent hæmolysis.

The monkey should next be chloroformed and fastened to an operating board. The tissues at the sides of the mouth may then be cut down and the jaws held open with a glass slide. The mouths of monkeys are usually in a very dirty condition and it is not surprising to find amœbæ and trichomonads in material obtained by scraping the gingival spaces at the base of the teeth with a small scalpel. This material should be spread out in saline solution under a cover glass. Trichomonads are conspicuous as they swim about with jerky movements; they can be seen with the low-power objective (16 mm). After some experience amœbæ can also be located under low magnification; their locomotion can best be observed with the high dry objective (4 mm) or under the oil-immersion lens. After the pre-

paration has been examined for living Protozoa the cover glass may be removed and the liquid allowed to evaporate until the smear is almost dry. Then it may be plunged into hot Schaudinn's solution and later stained with iron hæmatoxylin. If too much liquid remains on the slide everything will be washed off when placed in the fixative.

The abdominal wall of the monkey should now be opened and the entire intestine removed, as well as the spleen, urinary bladder, and vagina, if the monkey is a female. If the spleen is enlarged or dark the monkey was probably suffering from malaria. A tissue smear from the spleen should be made, dried, and cleared with immersion oil; this is sufficient to reveal the presence of the pigment granules characteristic of malaria. Trichomonads have been recorded from the vagina of monkeys (Hegner and Ratcliffe, 1927) and have been found by us in the vagina of Philippine monkeys. The inner wall of the vagina should be scraped gently and the material examined in saline solution on a slide. Trichomonads have also been recorded from the urinary bladder of men, but never from this organ in monkeys. They should be looked for, however, since it seems probable that they will eventually be found in the monkey.

A large number of Protozoa should be expected in the intestine. The duodenum is the optimum habitat of flagellates of the genus *Giardia;* these organisms and their cysts should also be searched for in the jejunum and ileum. A number of investigators have reported giardias from the bile ducts and gall bladder. These locations should be examined in monkeys that are found to be infected with these flagellates.

Another type of protozoan that lives in the small intestine of several of the lower animals is the coccidium. Coccidia have been reported from man in about two hundred cases but never from monkeys. Only the stages that occur in the oöcyst of *Isospora hominis* from man are known. We must construct the stages within the human body from what we know of the life cycle of *Isospora felis* in cats. If coccidia occur in monkeys they will eventually be found, and an opportunity will then be afforded to determine the stages that take place in the internal organs. The most conspicuous stage is the oöcyst, which is a body easily recognized and sufficient to indicate an infection. Care should be observed, however, not to accept oöcysts as evidence of infection until it has been determined that the oöcysts were produced within the body of the monkey and are

not parasites of other animals that had been swallowed by the monkey and were on their way through the intestine.

The large intestine of man and of the monkey furnishes a habitat for amœbæ, flagellates, and ciliates. Five types of amœbæ apparently belonging to species living in man have been recorded from monkeys; these are *Endamœba histolytica, E. coli, Endolimax nana, Iodamœba williamsi,* and *Dientamœba fragilis.* One or several of these are usually to be found in every monkey examined. In some cases the identity of the amœba can be determined from the living specimen, but often smears must be fixed and stained and the nuclear structure determined before the species is certain. Trophozoites seem to be most numerous in the cæcum, and cysts in the posterior part of the colon. The number of nuclei in cysts can be determined by adding a drop of a 5 per cent aqueous solution of potassium iodide saturated with iodine to the material under the cover glass. This stains the nuclei. One human intestinal amœba, *Dientamœba fragilis,* which has not been heretofore reported from monkeys, was found by us in two individuals. It has been recorded in less than one hundred human cases and is evidently rare in man as well as in monkeys.

The flagellates that are recognized as inhabitants of the large intestine of man have also been recorded from monkeys. These include *Trichomonas hominis, Chilomastix mesnili, Embadomonas intestinalis,* and *Tricercomonas intestinalis.* Trophozoites are more likely to be found in the cæcum than in the colon. All but *Trichomonas hominis* are known to form cysts; these should be sought in the lower colon.

A single species of ciliate, *Balantidium coli,* occurs in the large intestine of man, and is supposed to be the same as the species that lives in pigs and monkeys. It seems to be more numerous in the appendix of the monkey than in any other region. Three species of ciliates of the genus *Troglodytella* have been reported from the intestines of chimpanzees and gorillas but have not been found in other species of monkeys or in man.

It seems evident from the above description that monkeys offer excellent material, especially for the study of intestinal Protozoa (including those occurring in the mouth and vagina). We have found that monkeys recently captured are as frequently and as heavily infected as are specimens that have been in captivity for long periods. Efforts have been made to determine by exact measurement whether the Protozoa of monkeys and man are morphologically alike. The results indicate that

they are (Hegner and Chu, 1930). Cross-infection experiments are necessary before it can be stated definitely that they really belong to the same species. Many of them have been grown in artificial media, in which they exhibit characteristics that are comparable to those of human species. In some cases the intestinal Protozoa of monkeys are too few to furnish satisfactory material for the permanent slides necessary for identification. It is suggested that samples of intestinal contents be injected into the rectum of parasite-free young chicks; certain Protozoa grow and multiply rapidly in the cæca of the chick, thus furnishing an abundance of material for study (Hegner, 1929).

The following are the Protozoa observed by us in forty-four wild monkeys and the number of individuals infected by each:

| | Monkeys infected. |
|---|---|
| *Endamœba histolytica* | 10 |
| *Endamœba coli* | 22 |
| *Endamœba gingivalis* | 37 |
| *Endolimax nana* | 22 |
| *Dientamœba fragilis* | 2 |
| *Giardia lamblia* | 6 |
| *Trichomonas hominis* | 37 |
| *Trichomonas buccalis* | 36 |
| *Trichomonas vaginalis* | 2 |
| *Chilomastix mesnili* | 15 |
| *Balantidium coli* | 8 |

Most of our material was studied in the living condition only, hence this list cannot be considered complete. In several monkeys small flagellates were observed that resembled *Embadomonas, Tricercomonas,* and *Hexamita.* No specimen that could definitely be determined as *Iodamœba* was observed, although some would probably have been found if prepared slides were made from all of the monkeys. No coccidium or blood-inhabiting species was encountered.

As noted in another paper (Hegner, 1928) the study of monkey Protozoa has brought out an interesting point of biological significance. When two species of animals are parasitized by the same species a close relationship between the hosts is assumed. If this is applied to the protozoan parasites of monkeys and man a very close relationship between these two hosts is evident. This similarity of protozoan parasites indicates close kinship of man and monkeys and adds another type of evidence that man and monkeys have descended from a common ancestor.

### PROTOZOA OF TARSIUS

Four living specimens of *Tarsius fraterculus* were obtained from Bohol Island. Every one of them had intestinal infections with amœbæ and trichomonads.

### PROTOZOA OF OTHER MAMMALS

Many species of both blood-inhabiting and intestinal Protozoa have been described from domestic, laboratory, and wild mammals. Our observations indicate that these mammals are parasitized by the same types of Protozoa in the Philippines as in other countries.

*Carabao.*—The carabao, or water buffalo, is the common draft animal in the Philippines; its milk is used for food and its flesh takes the place of beef among many of the Filipinos. Protozoa, particularly ciliates, are abundant in the first and second stomachs (rumen and reticulum) of the carabao just as they are in cattle in other countries. We obtained material containing enormous numbers from carabaos slaughtered in Manila. No attempt was made to determine species, since this would require more time than was at our disposal. Becker and Talbott (1927) have reported on the Protozoa in the stomach of cattle and list two species of amœbæ, five species of flagellates, and thirty-nine species and varieties of ciliates. Besides these, many species of Protozoa have been recorded from the intestine of cattle including amœbæ, *Giardia, Trichomonas, Balantidium,* and coccidia. Among the blood-inhabiting Protozoa reported from cattle are babesias, *Plasmodium,* and trypanosomes. Sarcosporidia have also been found in the muscle of cattle. We did not examine the blood and intestine of the carabao for Protozoa. This animal offers attractive material for study.

*Pig.*—One of the most interesting protozoan parasites of the pig is the ciliate *Balantidium coli.* This species was found in the large intestine of pigs slaughtered in Manila. It also lives in the wild pigs that occur in the forests of the Philippines, as was demonstrated by the presence of many specimens in the one host available for study. We did not determine whether the balantidia of the domestic pigs of Manila and of the wild pig are of the *coli* type, *suis* type, or of both types (McDonald, 1922). Pigs are known to be infected with various species of intestinal amœbæ, flagellates, and coccidia, with blood-inhabiting trypanosomes and babesias, and with muscle sarcosporidia.

*Dog.*—Portions of the digestive tract of twelve dogs obtained from the department of physiology were examined by us. *Giardia canis* was found in the duodenum of one specimen and trichomonad flagellates in the large intestine of another specimen. No protozoan was noted in the mouth of four of the dogs and in the vagina of two. A more thorough study would probably reveal many more species, since dogs are known to harbor intestinal amœbæ, flagellates, and coccidia as well as blood-inhabiting babesias, leishmanias, and trypanosomes, and tissue-inhabiting *Sarcocystis.*

*Rabbits.*—Amœbæ, flagellates, and coccidia have been reported from rabbits. The amœba *Endamœba cuniculi* does not appear to be commonly present. Among the flagellates described by various investigators are *Giardia duodenalis, Chilomastix cuniculi, Trichomastix cuniculi, Embadomonas cuniculi,* and *Enteromonas intestinalis.* The most frequently present of all rabbit Protozoa are the coccidia. These are tissue-invading organisms and thus pathogenic, often bringing about the death of the host.

Laboratory rabbits maintained by the College of Medicine, University of the Philippines, were found to be well infected with coccidia. Rabbits are inhabited by at least two species of these Protozoa; namely, *Eimeria stiedae,* in the tissues of the liver, and *E. perforans,* in the intestinal wall. The fæcal pellets of infected rabbits should be softened in water and a small amount placed on a slide for examination. The oöcysts of *Eimeria stiedae* are ellipsoid or ovoid, flattened at one pole, and usually from 35 to 40 microns long and 23 to 28 microns broad. Stages in the formation of four sporoblasts within each oöcyst and of two sporozoites within each sporoblast may be observed in living specimens, if infected fæcal material is kept moist for a few days. If material is placed in a 5 per cent solution of potassium bichromate the growth of fungi and bacteria is retarded. Stages in the asexual cycle of *E. stiedae* may be obtained for study by fixing, sectioning, mounting, and staining tissue from the liver of an infected rabbit.

There is evidence in the literature on the coccidia of the rabbit that more than two species may be present (Kessel and Junks, 1929). In the fæcal pellets studied by us what appeared to be three types of oöcysts were present. One type resembles *Eimeria stiedae* in shape and size (Plate 1, figs. 4, 5, and 6). They were oval and averaged 35 microns in length and 23 microns

in breadth. The micropyle was conspicuous and broad. A second type appeared to be *E. perforans* (figs. 7, 8, and 9). Its oöcysts were also oval; they averaged 24 microns in length and 16 microns in breadth. The micropyle is not well marked, there being only a slight thinning of the wall at one end. The sporoblasts are at first spherical but later, when the sporozoites develop, become elongated. A spherical residual body is present. The third type (figs. 1, 2, and 3) is narrower at one end than at the other, has a well-defined but narrow micropyle, and is intermediate in size between the other two. It averages 31 microns in length and 19 microns in breadth. Four sporoblasts are formed and a residual body occurs. This type was more abundant than either of the other two in our material. Besides coccidia, *Giardia* and *Trichomastix* were encountered in the duodenum and colon, respectively, of the rabbits we examined.

*Guinea pig.*—Among the intestinal Protozoa that are frequently present in guinea pigs are flagellates, ciliates, amœbæ, and coccidia. *Giardia caviae* may be found in the duodenum, and the other species in the cæcum. Trichomonads are usually present in large members; *Chilomastix intestinalis* is a well-known species; *Embadomonas* has been described by several investigators; *Balantidium caviae* sometimes occurs in the cæcum (Scott, 1927); *Eimeria caviae* is fairly common in the large intestine (Sheather, 1924); *Endamœba cobayae* is a *coli*-like amœba with eight-nucleated cysts (Holmes, 1923) that may be encountered in the cæcum or colon; and *Endolimax caviae* (Hegner, 1926) is likewise an inhabitant of the cæcum. Infection with intestinal Protozoa evidently takes place among guinea pigs as a result of association, since if one member of a group that have lived together is infected with a certain species the other members are usually infected also.

The cæcum of guinea pigs is often very heavily infected with *Trichomonas caviae* Davaine, 1875. This species is one of the best for study because of its large size; many specimens in the cæcum of the two guinea pigs examined by us measured up to 25 microns in length and 12 microns in breadth. Enormous variation occurs in the size of guinea-pig trichomonads; specimens as small as 8 microns in length and 4 microns in breadth were also abundant. Whether more than one species are represented in guinea pigs is still in doubt. Faust (1921) believes he found differences of specific rank among trichomonads from

guinea pigs in Peking and described a species as *T. flagelliphora*. Cysts have been described and what appeared to be cysts were seen by the writers in the colon of the two guinea pigs examined. Whether more than one species of *Trichomonas* occur in guinea pigs is a problem that requires detailed cytological and experimental study. Perhaps the experimental infection of chicks would be helpful in determining this point (Hegner, 1929).

*Rat.*—Laboratory rats are of great value to protozoölogists as sources of material, as experimental animals, and as reservoirs for the maintenance of certain types of Protozoa. Among the intestinal Protozoa the following can almost always be obtained if half a dozen rats are examined: *Giardia muris* in the duodenum, *Hexamita muris* in the ileum, and *Trichomonas muris*, *Chilomastix bettencourti*, and *Endamœba muris* (fig. 53) in the cæcum or colon. Wild rats do not seem to be as highly parasitized with Protozoa as laboratory rats, but one of the best known of the trypanosomes, *T. lewisi*, occurs in them and is easily transferred to the latter. The laboratory rats that we have examined in Manila were found to contain specimens of all of the intestinal Protozoa listed above. No study was made of wild rats.

*Civet cat.*—Civet cats are common in certain regions of the Philippine Islands and can be obtained alive from the natives. Fæces collected by us in paths in the forest were found to contain large numbers of amœbæ and flagellates, probably coprozoic species. One living animal was anæsthetized in the laboratory; no blood-inhabiting protozoan was found in it, but giardias and coccidia were discovered in the duodenum. The giardias, when stained, obviously differed in specific characteristics from those that have been reported from other animals and are being studied in detail and will be fully described in a separate publication (Chu, 1930).

*Bat.*—Bats are abundant in the Philippines, and many species are available for study. They are not very heavily parasitized. This is probably due, so far as intestinal Protozoa are concerned, to the fact that they are continuously on the wing when away from their resting places, and, even when they are at rest, their fæces drop to the ground where their bodies never come in contact with them; thus contamination by contact with fæcal material must be infrequent and hence transmission difficult. Blood-inhabiting Protozoa, which are transmitted by blood-sucking arthropods, are no doubt frequently inoculated into bats

by their intermediate hosts. Therefore, it is not surprising that bats are more often reported to be infected with babesias, malarial parasites, and trypanosomes than with intestinal Protozoa. We collected bats from buildings in Manila and from caves and buildings within a radius of about 75 kilometers of Manila. Specimens were obtained from Paete, Teresa, Talisay, Sibul Springs, and Imus. These included the large fruit bat and small bats, both fruit bats and insectivorous bats. Over forty specimens were examined but no protozoan parasites were observed in the blood, and the only intestinal Protozoa found were coccidia of the genus *Eimeria* obtained from two specimens of an insectivorous species collected in a cave near Sibul Springs; ten specimens of this parasite ranged from 19 to 21 microns in length and from 17 to 20 microns in breadth; the average size was 20.7 by 18.9 microns. Plate 1, fig. 10, represents an oöcyst with a large residual body; fig. 11 was drawn one week later, at which time the residual body had almost entirely disappeared and the spores had become more fully developed.

*Flying lemur.*—Two living specimens of *Cynocephalus volans* were obtained from Bohol Island. The cæcum contained at least two species of flagellates; these were not studied in detail.

## PROTOZOA OF BIRDS

Both domesticated and wild birds are frequently infected with blood-inhabiting and intestinal Protozoa. The Protozoa of the domestic fowl, pigeon, duck, goose, guinea hen, etc., have not been studied very carefully, but amœbæ, flagellates, and Sporozoa have been described from them. The Protozoa of wild birds are very little known but would well repay investigation. Of the domesticated birds we have recorded Protozoa from the fowl and pigeon. It was our intention to use chicks as "living test tubes" (Hegner, 1929) in this and in other phases of our work, but difficulties in obtaining a sufficient supply prevented it. The wild birds examined by us were, with few exceptions, shot in the field. Blood films were made at once but only in about half of the cases were examinations of the intestinal contents made. A list of the wild birds and of the types of Protozoa found in them is given in Table 1.[3]

[3] We are indebted to Mr. Richard C. McGregor, of the Bureau of Science, for identifying many of these species for us. The family and species names have been taken from McGregor's Manual of Philippine Birds, Manila (1909).

TABLE 1.—*Wild Philippine birds and the Protozoa found in them.*

| Family and species. | Common name. | Date. | Locality. | Protozoa found. |
|---|---|---|---|---|
| TRERONIDÆ | | *1929* | | |
| *Osmotreron axillaris* | Philippine green pigeon | Aug. 24 | Cavite | None. |
| *Phapitreron leucotis* | Northern white-eared pigeon. | Aug. 24 | do | Do. |
| *Geopelia striata* | Barred ground dove | Aug. 24 | do | Do. |
| Do | do | Sept. 6 | Obando | Do. |
| *Leucotreron leclancheri* | Black-chinned fruit pigeon. | Oct. 25 | Dasmariñas | Do. |
| RALLIDÆ | | | | |
| *Gallinula chloropus* | Moorhen | Sept. 3 | Pateros | Do. |
| LARIDÆ | | | | |
| *Sterna sinensis* | White-shafted tern | Oct. 11 | Paete | Do. |
| Do | do | Oct. 11 | do | Do. |
| Do | do | Oct. 11 | do | Do. |
| Do | do | Oct. 11 | do | Do. |
| CHARADRIIDÆ | | | | |
| *Aegialitis alexandrina* | Kentish plover | Oct. 4 | Rosario | *Trichomonas.* |
| *Actitis hypoleucos* | Common sandpiper | Sept. 6 | Obando | Do. |
| *Gallinago stenura* | Pintail snipe | Oct. 11 | Paete | Do. |
| ARDEIDÆ | | | | |
| *Bubulcus coromandus* | Indian cattle egret | Sept. 17 | Talisay | *Giardia.* |
| Do | do | Sept. 20 | Marilao | Do. |
| Do | do | Sept. 20 | do | Do. |
| Do | do | Oct. 22 | Lemery | None. |
| Do | do | Oct. 22 | do | Do. |
| *Ixobrychus sinensis* | Little yellow bittern | Sept. 3 | Pateros | *Haemoproteus.* |
| Do | do | Oct. 11 | Paete | Do. |
| Do | do | Sept. 6 | Obando | Do. |
| *Ixobrychus cinnamomeus* | Cinnamon bittern | Sept. 13 | Angono | Do. |
| FALCONIDÆ | | | | |
| *Accipiter manillensis* | Philippine sparrow hawk | Sept. 17 | Talisay | Do. |
| *Haliastur intermedius* | Malayan Brahminy kite | Oct. 16 | Lubao | Coccidia. |
| Do | do | Sept. 17 | Talisay | Do. |
| *Microhierax erythrogenys* | Philippine falconet | Oct. 25 | Dasmariñas | Do. |
| PSITTACIDÆ | | | | |
| *Bolbopsittacus lunulatus* | Luzon guaiabero | Aug. 24 | Cavite | Do. |
| CORACIIDÆ | | | | |
| *Eurystomus orientalis* | Broad-billed roller | Sept. 26 | Dasmariñas | *Haemoproteus.* |
| Do | do | Sept. 24 | Arayat | None. |
| ALCEDINIDÆ | | | | |
| *Halcyon coromandus* | Ruddy kingfisher | Sept. 24 | do | Coccidia. |
| *Halcyon gularis* | White-throated kingfisher | | | |
| *Halcyon chloris* | White-collared kingfisher | Sept. 13 | Angono | None. |
| Do | do | Sept. 13 | do | Do. |
| Do | do | Sept. 6 | Obando | Do. |
| *Halcyon lindsayi* | Lindsay's kingfisher | Sept. 14 | Talisay | *Haemoproteus.* |
| Do | do | Sept. 24 | Arayat | None. |

TABLE 1.—*Wild Philippine birds and the Protozoa found in them*—Ctd.

| Family and species. | Common name. | Date. | Locality. | Protozoa found. |
|---|---|---|---|---|
| BUCEROTIDÆ | | *1929* | | |
| *Penelopidis manillae* | Luzon tarictic | Sept. 17 | Talisay | None. |
| MEROPIDÆ | | | | |
| *Merops philippinus* | Green-headed bee-bird | Oct. 11 | Paete | Do. |
| MICROPODIDÆ | | | | |
| *Collocalia troglodytes* | Pigmy swiftlet | Sept. 14 | Talisay | Do. |
| Do | do | Sept. 14 | do | Do. |
| *Collocalia marginata* | Salvadori's swiftlet | Sept. 26 | Dasmariñas | Do. |
| *Tachornis pallidior* | Paler palm swift | Sept. 14 | Talisay | Do. |
| CUCULIDÆ | | | | |
| *Centropus javanicus* | Java coucal | Aug. 24 | Cavite | *Haemoproteus.* |
| PICIDÆ | | | | |
| *Yungipicus validirostris* | Large-billed pigmy woodpecker. | Sept. 26 | Dasmariñas | None. |
| *Lichtensteinipicus funebris.* | Funereal woodpecker | Sept. 14 | Talisay | Do. |
| HIRUNDINIDÆ | | | | |
| *Hirundo gutturalis* | Eastern swallow | Oct. 14 | Rosario | Do. |
| CAMPOPHAGIDÆ | | | | |
| *Edolisoma caerulescens* | Luzon cuckoo shrike | Sept. 24 | Arayat | Do. |
| *Lalage niger* | Pied lalage | Sept. 24 | do | Coccidia. |
| Do | do | Sept. 24 | do | Do. |
| PYCNONOTIDÆ | | | | |
| *Iole gularis* | Philippine bulbul | Oct. 30 | Dasmariñas | *Haemoproteus.* |
| Do | do | Oct. 1 | Teresa | Do. |
| Do | do | Oct. 1 | do | Do. |
| Do | do | Sept. 14 | Talisay | *Plasmodium.* |
| Do | do | Sept. 9 | Dasmariñas | None. |
| *Pycnonotus goiavier* | Guava bulbul | Oct. 1 | Teresa | Do. |
| Do | do | Aug. 24 | Cavite | Do. |
| TURDIDÆ | | | | |
| *Practincola caprata* | Pied chat | Aug. 23 | Novaliches | Do. |
| Do | do | Aug. 23 | do | Do. |
| LANIIDÆ | | | | |
| *Cephalophoneus nasutus* | Large-nosed shrike | Sept. 13 | Angono | *Plasmodium.* |
| Do | do | Sept. 13 | do | None. |
| Do | do | Aug. 24 | Cavite | Do. |
| *Otomela lucionensis* | Gray-headed shrike | Oct. 30 | Dasmariñas | Coccidia. |
| Do | do | Aug. 6 | Novaliches | Coccidia, *Plasmodium.* |
| Do | do | Aug. 6 | do | Coccidia. |
| Do | do | Aug. 6 | do | Do. |
| Do | do | Aug. 6 | do | Do. |
| Do | do | Aug. 6 | do | Do. |
| Do | do | Aug. 6 | do | Do. |
| Do | do | Sept. 13 | Angono | None. |
| Do | do | Sept. 20 | Sibul springs | Do. |

TABLE 1.—*Wild Philippine birds and the Protozoa found in them*—Ctd.

| Family and species. | Common name. | Date. | Locality. | Protozoa found. |
|---|---|---|---|---|
| MOTACILLIDÆ | | *1929* | | |
| *Motacilla melanope* | Streak-eyed wagtail | Oct. 4 | Rosario | None. |
| Do | do | Oct. 4 | do | Do. |
| Do | do | Sept. 26 | Dasmariñas | Do. |
| ALAUDIDÆ | | | | |
| *Passer montanus* | Mountain sparrow | Aug. 13 | Manila | Coccidia. |
| Do | do | Aug. 13 | do | Do. |
| Do | do | Aug. 13 | do | Do. |
| Do | do | Aug. 13 | do | Do. |
| Do | do | Aug. 13 | do | None. |
| PLOCEIDÆ | | | | |
| *Padda oryzivora* | Java sparrow | Aug. 23 | Paombong | Do. |
| Do | do | Aug. 23 | do | Do. |
| Do | do | Aug. 26 | Muntinlupa | Coccidia. |
| Do | do | Aug. 26 | do | None. |
| *Munia jagori* | Philippine weaver | Aug. 23 | Paombong | Do. |
| Do | do | Aug. 23 | do | Do. |
| Do | do | Sept. 3 | Pateros | Do. |
| *Munia cabanisi* | Cabanis's weaver | Sept. 3 | do | Do. |
| ORIOLIDÆ | | | | |
| *Oriolus acrorhynchus* | Philippine oriole | Sept. 9 | Dasmariñas | *Haemoproteus.* |
| Do | do | Sept. 9 | do | Do. |
| Do | do | Sept. 9 | do | None. |
| STURNIDÆ | | | | |
| *Sturnia sinensis* | Gray-backed starling | Oct. 25 | Dasmariñas | *Trichomonas*, Coccidia. |
| Do | do | Oct. 25 | do | Do. |
| Do | do | Oct. 25 | do | Do. |
| *Æthiopsar cristatellus* | Crested myna | Sept. 24 | Arayat | Do. |
| *Sarcops calvus* | Gray-backed coleto | Oct. 15 | Dasmariñas | *Haemoproteus.* |
| CORVIDÆ | | | | |
| *Corone philippina* | Philippine crow | Sept. 17 | Talisay | None. |

*Blood-inhabiting Protozoa of birds.*—Protozoa of the genera *Hepatozoon, Haemoproteus, Leucocytozoon, Plasmodium, Toxoplasma,* and *Trypanosoma* have been recorded from the blood of birds. Those most frequently present belong to the genera *Haemoproteus* and *Plasmodium.*

*The genus Haemoproteus.*—Species belonging to this genus have been reported from large numbers of birds and from turtles and lizards. The fact that they grow around the nucleus of the red cell and become shaped like a halter, instead of pushing the nucleus to one side as do the parasites of bird malaria, prompted Labbé (1894) to propose the genus name *Halteridium* for this type. However, the name *Haemoproteus* has priority.

The best-known species is *Haemoproteus columbae* of the pigeon. The fly *Lynchia maura* is the transmitting agent of this species, and developmental stages similar to those of the malarial organism in mosquitoes occur in this fly. Other agents must be responsible for transmission to pigeons in certain areas and to other birds since the parasites occur in birds that live in areas where *Lynchia maura* does not exist. It is of interest to note that the observation of MacCallum (1897) of the formation of microgametes and subsequent fertilization of macrogametes of *Haemoproteus* led him (MacCallum, 1898) to look for and find a similar process in the gametocytes of human malaria.

*Haemoproteus* was found in the blood of seven of the forty-seven species and ninety-five individual birds examined by us as shown in the accompanying list. Unlike malarial parasites, no asexual stages occur in the peripheral blood, this stage in the life cycle being passed through in the endothelial cells of the blood vessels. Thus only gametocytes are to be found in blood films. How many species of *Haemoproteus* are represented in our material has not been determined. Plate 2, figs. 19 to 35, shows camera lucida drawings of a few of the parasites and gives some idea of their characteristics.

*The genus Plasmodium.*—Species of the genus *Plasmodium* have been described from birds in various parts of the world, and some of the most important work on malaria has resulted from the study of this disease in birds.

How many species of *Plasmodium* occur in birds is not known. For many years the name *Plasmodium praecox* was employed to designate all parasites of this type reported from birds. Recently, however, Hartman (1927) has recognized three distinct species that occur in English sparrows in the eastern United States, and probably other species of *Plasmodium* exist in this and other species of birds. Bird malarial organisms are favorable for experimental studies because they can be inoculated easily into canaries. Canaries grown in captivity are free from malarial parasites. They can be inoculated easily by drawing into a small syringe containing a little normal saline solution, a few drops of blood from an infected bird and injecting this into the breast muscle or peritoneal cavity of a fresh canary. As a rule parasites begin to appear in the blood of this bird in about five days; the maximum number of parasites are present five days later and parasites disappear from the blood at the end of about five days more. That parasites are still present in the

blood is evident since the blood of a bird at this stage when inoculated into a fresh bird will bring about an infection. They are so few in number, however, that they cannot be found by routine methods of examination but only after exhaustive searching. Apparently, in most cases, a bird once infected remains infected throughout its life (Hegner, 1929).

The characteristics that have been used to distinguish species of bird malarial organisms are length of the asexual cycle; presence or absence of schizonts in the peripheral blood; shape and size of the gametocytes and trophozoites; staining reactions of the gametocytes and trophozoites; the number, size, shape, and distribution of pigment granules; the number of merozoites; and the number of parasites per given number of red blood corpuscles (see Hartmen, 1927).

Malarial parasites were found in only three birds. Typical specimens are illustrated on Plate 3, figs. 39 to 46. The species found in the gray-headed shrike (fig. 45) seems to have larger pigment granules than any heretofore recorded for *Plasmodium* in birds, but we are not prepared at present to pronounce it a new species. The small number of infections found (3 in 95 birds) appear to indicate a low incidence of infection. However, it should be remembered that the acute stage of the infection lasts for only a few days (about ten days in the canary) and that after the end of the acute stage so few parasites remain in the blood stream that they are extremely difficult tc find. It seems probable, therefore, that many infections were not found. Perhaps the inoculation of blood from these birds into parasite-free canaries would have revealed infections that escaped discovery. This method should be employed in any intensive study of this subject.

*Intestinal Protozoa of birds.*—Various species of amœbæ, flagellates, and coccidia live in the intestines of birds. Among those most frequently encountered are trichomonads and coccidia. The coccidia penetrate the intestinal wall and develop at the expense of the tissue cells. Their presence is indicated by the discovery of oöcysts in the fæces. Several species of coccidia may inhabit a single individual, the species differing in their distribution within the tissues of the digestive tract (Tyzzer, 1929), being located in the duodenum or cæcum or colon. The amœbæ and flagellates of birds are mostly restricted to the cæca and are therefore to be found most frequently in birds that possess well-developed cæca.

*Protozoa of fowls.*—Intestinal Protozoa of birds are most easily obtained from domestic fowls. Two species of trichomonads were described from fowls by Martin and Robertson (1911); namely, *Trichomonas eberthi* with three anterior flagella, and *T. gallinarum* with four anterior flagella. These investigators also described *Chilomastix gallinarum. Endamœba gallinarum* was reported from fowls by Tyzzer (1920) and *Endolimax gregariniformis* by Tyzzer (1920) and by Hegner (1926). These are all inhabitants of the cæcum. Coccidia have been known to occur in fowls for over seventy years and are of common occurrence. The name *Eimeria avium* has been applied to these coccidia, but the recent work of Tyzzer (1929) indicates that the domestic fowl may be parasitized by as many as three species.

We did not attempt to make an exhaustive study of fowl Protozoa. Two young pullets were examined; the cæca of one contained an abundance of trichomonads, a few individuals of *Chilomastix,* and a few of *Endamœba;* the cæca of the other were almost empty, but numerous trichomonads were present, a few specimens of *Endolimax,* and a few coccidia. The trichomonads varied considerably in size; it was impossible to distinguish species from the living specimens, but both of the species recognized by Martin and Robertson may have been present. The *Chilomastix* was probably *C. gallinarum.* The *Endamœba* corresponded in size and characteristics to *E. gallinarum;* it measured in the living condition about 28 microns in length and 20 microns in breadth (Plate 4, fig. 50). The *Endolimax* resembled *E. gregariniformis;* it was about 15 microns long and 7 microns broad (fig. 52). The oöcysts of the coccidia found were unsegmented; they no doubt belonged to the genus *Eimeria.* One may confidently expect to find specimens of *Trichomonas, Chilomastix, Endamœba, Endolimax,* and *Eimeria* in the cæca of fowls if several birds are examined.

*Pigeon.*—The pigeon is the host of a coccidium named *Eimeria pfeifferi,* and a high incidence of infection has been noted. One pigeon that we examined contained large numbers of oöcysts. Measurements of fifty specimens are presented in Table 2.

Table 2 shows a high degree of correlation between length and breadth. These oöcysts are smaller than those described by Nieschulz (1921), who gives their size as 14 to 24 microns in breadth and 15 to 26 microns in length.

*Wild birds.*—No amœbæ and only two types of flagellates were found in the intestines of the wild birds we examined.

TABLE 2.—*Correlation of measurements of fifty oöcysts of Eimeria pfeifferi from a pigeon.*

| Breadth of oöcysts in microns. | Length of oöcysts in microns. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 13 | 14.3 | 15.6 | 16.9 | 18.2 | 19.5 | |
| 11.7 | 2 | 1 | | | | | 3 |
| 13.0 | 4 | 3 | 4 | | | | 11 |
| 14.3 | | 4 | 4 | 2 | 1 | | 11 |
| 15.6 | | | | 9 | 5 | 1 | 15 |
| 16.9 | | | | 5 | 3 | 2 | 10 |
| Total | 6 | 8 | 8 | 16 | 9 | 3 | 50 |

*Trichomonas* occurred in the Kentish plover, common sandpiper, and gray-backed starling, and *Giardia* was present in three of five specimens of the Indian cattle egret. No detailed study was made of the trichomonads. The giardias of birds are taxonomically in a confused state at present (Hegner, 1925).

Wild birds are not as heavily infected with intestinal Protozoa as are domestic fowls. Those most frequently encountered are coccidia of the genera *Eimeria* and *Isospora*. The list of birds that we examined indicates only partially to what extent one may expect to find coccidia in wild birds, since not more than half of these birds were examined for coccidia. Seven species were positive. Apparently those most often infected are the pied lalage, gray-headed shrike, mountain sparrow, and gray-backed starling. Some of the intestinal material containing coccidia was placed in potassium bichromate solution and their development followed. All proved to belong to the genus *Isospora*, but we made no attempt to determine whether or not we were dealing with new species. The oöcysts from the crested myna (Plate 1, fig. 14) were almost spherical and contained two pear-shaped spores and a very small residual body. Ten specimens ranged from 22 to 27 microns in diameter; the average diameter was 23.8 microns. A slight bulge where the oöcyst wall was a little thinner than elsewhere indicates the position of the micropyle. The oöcysts from the pied lalage were oval and also contained two pear-shaped spores and a small residual body (fig. 15). The position of the micropyle at one end could be determined by the thinner wall of the oöcyst. Ten specimens ranged from 21 to 24 microns in breadth and from 24 to 28 microns in length with an average breadth of 22.9 microns and an average length of 27.5 microns. What are probably two species were found in the gray-backed starling. One species

was oval and about 23 microns in diameter, and the other oval and about 21 microns broad and 27 microns long. No residual mass was noted in the oöcyst. The oöcysts found in the brahminy kite were of various shapes due to the thinness of the wall and different positions of the spores within. Three specimens measured 13 by 18 microns, 16 by 18 microns, and 12 by 21 microns, respectively. In the ruddy kingfisher were found spores with a knoblike process at either end. They measured 13 by 25 microns. An immature spore is shown in fig. 12 and a spore drawn six days later is shown in fig. 13, both on Plate 1.

### PROTOZOA OF REPTILES

Reptiles of various types, turtles, snakes, lizards, and crocodiles are inhabited by various species of both blood-inhabiting and intestinal Protozoa. We have examined only a few specimens of Philippine reptiles. In every case the blood was negative, but in many specimens intestinal Protozoa were found.

*Turtle.*—One specimen of *Cyclemys amboinensis* that had been in captivity in Manila was free from Protozoa.

*Python.*—One python, *Python reticulatus,* captured at Montalban had trichomonads and coccidia (Plate 1, figs. 16 and 17) in the intestine. The trichomonads were cultivated successfully at room temperature in serum-saline-citrate medium and positive subcultures obtained.

*Iguana.*—Six specimens of the species *Hydrosaurus pustulosus* were shot at Talisay. Every one was found to contain trichomonads in the intestine.

*Lizards.*—All of five lizards of the species *Mabuya multicarinata* had both trichomonads and coccidia in the intestine.

### PROTOZOA OF AMPHIBIA

*Frogs.*—Frogs are well supplied with Protozoa. Among the commoner blood-inhabiting species are trypanosomes, hæmogregarines, and *Cytamœba.* The intestinal species that are frequently present belong to the genera *Balantidium, Endamœba, Hexamita, Nyctotherus, Opalina,* and *Trichomonas.* Other genera are not encountered as often. It is thus evident that frogs are important animals from which to obtain material for study and research. *Trypanosoma rotatorium,* which occurs in the blood plasma, is the type species of its genus. The opalinids are large ciliates that are of particular interest because of their use by Metcalf (1923) for studies of host relationships. A number of species of *Balantidium* have been described from

frogs from various parts of the world. The trichomonads of frogs fix and stain beautifully which makes them useful for students, since species from certain other animals, including man, can be well stained only with difficulty. Frog tadpoles are also usually heavily infected with intestinal Protozoa. Many of these are of the same species as those living in the adults, but both *Giardia agilis* and *Euglenamorpha hegneri,* occur in tadpoles only, and in certain species of frogs the opalinids are lost at the time of metamorphosis. An ectoparasitic ciliate, *Trichodina pediculus,* sometimes may be found on the surface of the body of tadpoles.

The Opalinidæ are among the ciliates most easily obtained for study. Most of them occur in the rectum of frogs, toads, and tadpoles. Material from the rectum of freshly killed animals usually contains large numbers of specimens. According to Metcalf (1923) four genera are included in the family Opalinidæ; these are *Protoopalina,* which is cylindrical and binucleate; *Zelleriella,* which is flattened and binucleate; *Cepedea,* with a cylindrical form and many nuclei; and *Opalina,* with a flattened form and many nuclei. In his monograph on the Opalinidæ Metcalf (1923) states that up to that time twenty-five species had been described, two of which were doubtful. He added one hundred twenty species, of which eighteen were somewhat doubtful; twenty subspecies, of which six were doubtful; and ten forms. Other species have been described since this monograph was published. Anyone who wishes to study the many fascinating problems presented by this group of ciliates should first consult the above-mentioned monograph as well as more-recent literature by Metcalf and others.

In Taylor's (1921) monograph on the amphibians and turtles of the Philippine Islands, sixty-six species of amphibians are described, which according to Taylor include probably not more than one-half of those that exist in the Islands. Among these there are only three species that are listed by Metcalf (1923) as hosts of opalinas; these are *Bufo melanostictus, Rana erythraea,* and *R. tigerina.* It is thus evident that a large field is open for students of the Opalinidæ in the Philippines. Opalinas may be obtained not only from freshly killed hosts but also from animals that have been preserved in alcohol for many years. A slit may be made in the abdomen of museum specimens of amphibians, the rectum pulled out and slit, and the contents of the rectum removed. The opalinids secured in this way are often well preserved, and even the nuclear structure and chromosome num-

ber can be determined in many cases. According to Metcalf a satisfactory method of preparing specimens for study is to make a smear on a cover glass, fix in Schaudinn's solution, and stain with Delafield's hæmatoxylin, overstaining and then reducing.

Criteria of value in separating species of opalinids are the following: Shape of body; length, breadth, and thickness of body; length and distribution of cilia; width between lines of cilia at the anterior end and near the posterior end of the body; relative amounts and structure of ectoplasm and endoplasm; number of nuclei, shape, size, position in the body and position with respect to one another; and structure and number of chromosomes in the nuclei. Taxonomic studies of opalinids are accompanied by various difficulties, such as racial diversities, lack of a satisfactory method of cultivation outside of the body, differences in size and structure due to physiological conditions and stage of life cycle, and the presence of two or more species in a single host.

Besides taxonomic studies, opalinids afford excellent material for the investigation of the relationships of hosts as indicated by the presence of identical or nearly allied ciliates of this group. Metcalf has shown how effective this type of research can be, but has not had access to material from the Philippine Islands and hence a large and interesting group remains to be studied.

Our studies of frog Protozoa were limited to those of two species of hosts; namely, fourteen specimens of *Rana vittigera* and one specimen of *Kaloula picta.* A considerable number of tadpoles were examined, but the species of frogs to which they belonged was not determined. Trypanosomes were found in the blood of only one specimen of *R. vittigera. Trichomonas* was present in the rectum of every frog examined, often in large numbers. *Hexamita* was also a frequent inhabitant of the rectum, being present in five specimens of *R. vittigera* and in the specimen of *K. picta. Balantidium* was encountered in every frog. A typical living specimen from *R. vittigera* shown in fig. 36 measured 81 microns in length and 52 microns in breadth. A typical specimen of another species from *K. picta,* shown on Plate 3, fig. 47, measured 130 microns long by 102 microns broad. Opalinas of several species were found in the rectum of six specimens of *R. vittigera;* some of them measured as much as 950 microns in length. Amœbæ of two types occurred in three specimens of *R. vittigera;* these corresponded in size, shape, and locomotion to *Endamœba ranarum* and *Endolimax ranarum*

(Plate 3, fig. 38). One specimen of *R. vittigera* was infected with *Chilomastix* and another with coccidia. The tadpoles examined were free from *Giardia* and *Euglenamorpha,* but all were infected with *Trichomonas, Opalina,* and amœbæ. Many algæ were noted in the intestines of these tadpoles, but whether any of them were parasites was not determined; most of them were probably free-living species ingested with the food material. No ectoparasites were found living on tadpoles.

## INTESTINAL PROTOZOA OF INSECTS

Many species of Protozoa have been described from the intestine of insects; they live either in the lumen of the intestine or in the tissues of the intestinal walls. Only a few common species of insects were examined by us; these included the housefly, cockroach, and mosquito.

*Housefly.*—The genus *Herpetomonas* is included in the family Trypanosomidæ. Its members are parasitic in invertebrates, mostly insects, and are transmitted from host to host in the cyst stage by the fæcal contamination of food. It has been the custom to propose a new specific name for every herpetomonad discovered in a new species of host regardless of morphological distinctions. Becker (1923) has shown by cross-infection experiments that the herpetomonads living in each of six different species of flies are infective to the other five and that probably all belong to one species.

The species most easily obtained is *Herpetomonas muscae domesticae,* the type species of the genus. It has been recorded from the intestine of houseflies in various parts of the world. The intestine of freshly killed flies should be teased out in normal saline solution. Permanent preparations may be made by fixing and staining smears by the Schaudinn iron-hæmatoxylin method or by the Giemsa method.

About 40 per cent of several dozen flies examined by us were found to be infected with *Herpetomonas.* Plate 5, fig. 56, shows some of the different types present.

*Mosquitoes.*—We are indebted to Dr. C. Manalang for specimens of anopheles mosquitoes containing coccidia (Plate 1, fig. 18). According to Manalang (1929) no records of coccidial infections of mosquitoes had been published previous to his account. Manalang found oöcysts in from 1 to 2 per cent of all the common species of adult anopheles from the Novaliches Water Project and the San Francisco malaria control areas near Manila, both in the spring of 1928 and of 1929. These

included *Anopheles minimus,* a carrier of human malaria, *A. vagus,* and *A. tessellatus.* He also noted oöcysts in the larva of *A. tessellatus.* Either two species or two stages in the life cycle of one species were observed. Certain oöcysts were brownish yellow and measured from 66 microns by 37 microns to 31 microns by 25 microns; the average was 44.8 microns by 29 microns. Others were colorless and slightly smaller, ranging from 58 microns by 31 microns to 25 microns by 24 microns; the average was 37.8 microns by 26.7 microns. Adults contained oöcysts almost everywhere throughout the body except the midgut, brain, and eggs. In the parasitized larva the oöcysts were distributed to all parts of the body. No developmental stages within the oöcysts were observed.

*Cockroaches.*—Cockroaches are common household insects, and their association with man makes them of special interest to protozoölogists because of the possibility that they may serve as vectors of human Protozoa. It does not seem probable that the Protozoa ordinarily present in the cockroach belong to the same species as those living in man, but material containing human Protozoa is no doubt often ingested by cockroaches, and if these Protozoa are able to pass through the digestive tract of the insect in a viable condition they might be deposited in the food or drink of man and thus bring about infection. Amœbæ and ciliates belonging to the same genera as those living in man commonly occur in cockroaches; these are *Endamœba* and *Balantidium.* It seems certain, however, that the species are different and that cross-infection rarely if ever takes place. Attempts to infect cockroaches with the trophozoites of certain human Protozoa have shown that these are unable to pass through the digestive tract without being destroyed (Hegner, 1928). Whether cysts can withstand the conditions in the stomach and intestine of the insects is still to be determined. The intestinal Protozoa that may usually be found in cockroaches are *Endamœba blattae,* a very large species; a smaller species of *Endamœba, E. thomsoni;* an *Endolimax, E. blattae;* two or more species of ciliates, *Balantidium blattarum* and *Nyctotherus ovalis;* several species of flagellates, including *Hexamita periplanetae, Lophomonas blattarum,* and *L. striata;* and *Retortomonas orthopterorum.* Besides these, half a dozen species of gregarines have been described from these insects.

Three species of cockroaches were examined by us, all captured in Manila, as follows: *Periplaneta americana,* six speci-

mens; *Nauphoeta cinerea,* three; and *Rhyporobia maderae,* two. Every specimen examined contained intestinal Protozoa but the species of cockroaches differed widely with respect to the species of Protozoa living in them, even when the hosts were collected from the same market. It is obviously not sufficient to state that a certain species of Protozoon occurs in the cockroach, but the species of cockroach should always be specified. The great difference in the intestinal fauna of different species of cockroaches living in the same environment suggests experimental studies on host-parasite specificity. Cockroaches can easily be freed from their intestinal Protozoa by the oxygenation method (Cleveland, 1925), and can then be fed easily on material from other species of cockroaches (Hegner, 1928).

The species present in the six specimens of *Periplaneta americana* that we examined were as follows: *Nyctotherus ovalis* (fig. 48), abundant in five specimens; *Endamœba thomsoni* (fig. 51), a few to many in five specimens; *Hexamita periplanetae,* present in all specimens; *Retortomonas orthopterorum,* present in three specimens; *Endamœba blattae* (Fig. 49), many in one specimen; *Lophomonas striata* (Fig. 54), and *L. blattarum* (Fig. 55) present in one specimen; gregarines, many in one specimen. These Protozoa were examined in the living condition only, no stained preparations being prepared. It is, therefore, impossible to state from a detailed study whether they differed specifically from the list as given above.

The three specimens of *Nauphoeta cinerea* examined were particularly characterized by the large number of gregarines present. At least three species of these could be distinguished. Besides gregarines, there were many amœbæ belonging apparently to several species, but no *Endamœba blattae,* and two or three species of very small flagellates.

*Rhyporobia maderae,* of which two specimens were examined, also contained many gregarines of several species. Flagellates, probably of the genera *Hexamita* and *Retortomonas,* were present in large numbers.

*Endamœba blattae* (Plate 4, fig. 49) is a very large amœba and hence of great value for classroom study. It is the type species of the genus *Endamœba;* it was described and named by Leidy in 1879. The nucleus is conspicuous in the living animal and has a heavy membrane. The cytoplasm is grayish, and no clear ectoplasm is visible. As the organism moves along, dark lines appear in the cytoplasm parallel to the direction of

locomotion. This species occurred in large numbers in the rectum of most of the specimens of the cockroach *Periplaneta americana* collected at the Divisoria Market, in Manila, but was absent from all of those obtained from one of the hospitals. It is advisable, therefore, when looking for *E. blattae,* to examine cockroaches from several different localities. The life cycle of *E. blattae* has been described in detail by Mercier (1910) but needs to be confirmed.

The following species of gregarines are listed by Watson (1916) from cockroaches: *Gregarina blattarum* from *Periplaneta orientalis, P. americana,* and *Blatella germanica; G. serpentula* from *Periplaneta orientalis;* and *Gamocystis tenax* from *Blatella lapponica.*

The criteria of use in distinguishing species of gregarines are "size, both medium and average; ratio of length of protomerite to total length; ratio of width of protomerite to width of deutomerite; general shape of the body; shape of the protomerite; shape of the deutomerite; character of the interlocking device; size and shape of the nucleus; color and character of the protoplasm; and size and shape of the cysts and their method of dehiscence." (Watson, 1916, p. 42.) Several types of gregarines found by us are probably undescribed species and would well repay detailed study.

### PROTOZOA OF SNAILS

Certain snails of the genus *Ampullaria* that are abundant in the fresh waters of the Philippine Islands are infected with *Balantidium haughwouti* de Leon (1919). A snail, *Melania blatta* Lea, that we collected in considerable numbers at San Francisco del Monte, was infected with balantidia, which may be *B. haughwouti.*

### PROTOZOA OF PLANTS

The interesting fact that parasitic Protozoa may live in plants as well as in animals was demonstrated by Lafont in 1909, who described a flagellate from the latex of a plant, *Euphorbia pilulifera,* on Mauritius. Since then flagellates have been reported from latex-bearing plants belonging to several families and from Europe, Asia, Africa, and North and South America. None have heretofore been reported, so far as we know, from the Philippine Islands. The generic name *Phytomonas,* proposed by Donovan in 1909, has been adopted by several protozoölogists for these flagellates, largely because of their peculiar habitat.

Holmes (1925) has probably furnished the best life-history studies of any member of the genus. He has described the species *P. elmassiani* from milkweed plants, *Asclepias syriaca,* in the eastern United States and has found these flagellates in what appears to be undoubtedly their intermediate host, a bug, *Oncopeltus fasciatus,* which feeds on milkweed plants. Within this plant, *P. elmassiani* is confined to the latex system and is intracellular but not intracytoplasmic. Certain latex cells may be infected and others not, within a single plant. The flagellates occur only in the salivary glands of the bug where they are localized in the dorsal and anterior lobes but do not occur in the posterior lobe of the gland.

*Phytomonas* seems to be pathogenic to certain euphorbias but does not seem to injure milkweeds. The true host-parasite relations of the genus are, however, still to be determined. Many species of Euphorbiaceæ and other latex-bearing plants occur in the Philippines. These would well repay careful examination. Suggestions regarding problems have been published recently by Holmes (see Hegner and Andrews, 1930).

We have examined the latex of a large number of species of plants growing in and around Manila and belonging to nine different families as listed below. These plants were obtained through the kindness of Dr. E. Quisumbing, of the Bureau of Science, Manila. As a rule, only one plant of each species was examined. The only species found to be infected was *Euphorbia hirta.* About 60 per cent of the plants contained flagellates, many of which remained alive in the latex within the plants for at least twenty-four hours after being brought into the laboratory. Several types of flagellates are shown on Plate 5, fig. 57.

TABLE 3.—*Plants examined for flagellates.*

Anacardiaceæ.
- *Mangifera indica* Linn.

Apocynaceæ.
- *Allamanda hendersonii* Bull.
- *Allamanda cathartica* Linn.
- *Plumiera acuminata* Ait.
- *Tabernaemontana divaricata* (Linn.) R. Br.
- *Tabernaemontana pandacaqui* Poir.
- *Thevetia peruviana* (Pers.) Merr.

Asclepiadaceæ.
- *Calotropis gigantea* (Linn.) Dryand.
- *Dischidia ruscifolia* Decne.
- *Dischidia vidalii* Becc.

Campanulaceæ.
- *Isotoma longiflora* (Mill.) Presl.

Caricaceæ.
- *Carica papaya* Linn.

Convolvulaceæ.
- *Ipomoea batatas* (Linn.) Poir.

Convolvulaceæ—Continued.
*Ipomoea cairica* (Linn.) Sweet.
*Ipomoea fistulosa* Mart.
*Ipomoea reptans* (Linn.) Poir.
Euphorbiaceæ.
*Euphorbia heterophylla* Linn.
*Euphorbia hirta* Linn.
*Euphorbia pulcherrima* Willd.
*Euphorbia splendens* Boj.
*Euphorbia tirucalli* Linn.
*Euphorbia trigona* Haw.
*Jatropha curcas* Linn.
*Manihot utilissima* Pohl.
*Melanolepis multiglandulosa* (Reinw.) Reichb. f. and Zoll.
Euphorbiaceæ—Continued.
*Pedilanthus tithymaloides* Poir.
*Phyllanthus reticulatus* Poir.
Moraceæ.
*Artocarpus communis* Forst.
*Artocarpus cumingiana* Trec.
*Artocarpus integra* (Thunb.) Merr.
*Ficus elastica* Roxb.
*Ficus hauili* Blanco.
*Ficus minahassae* (Teysm. and De Vr.) Miq.
*Ficus nota* (Blanco) Merr.
*Ficus religiosa* Linn.
*Morus alba* Linn.
Sapotaceæ.
*Chrysophyllum cainito* Linn.

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# ILLUSTRATIONS

## Plate 1. Coccidial Oöcysts from Philippine Animals

Figs. 1 to 3. Three stages in the development of oöcysts of a species of *Eimeria* from the rabbit.

4 to 6. Stages in the development of the oöcyst of another species of *Eimeria* from the rabbit.

7 to 9. Stages in the development of the oöcyst of a third species of *Eimeria* from the rabbit.

10 and 11. Two stages in the development of the oöcyst of an *Eimeria* from a bat.

12 and 13. Two stages in the development of an *Isospora* from the ruddy kingfisher, *Halcyon coromandus.*

Fig. 14. An oöcyst of *Isospora* from the crested myna, *Æthiopsar cristatellus.*

15. An oöcyst of *Isospora* from the pied lalage, *Lalage niger.*

Figs. 16 and 17. Two stages in the development of an *Eimeria* from the python.

Fig. 18. An oöcyst from a larval stage of a mosquito, *Anopheles tessellatus.*

All figures of Plate 1 were drawn with a camera lucida from living specimens; they are all magnified 1000 diameters except fig. 18 which is magnified 250 diameters.

## Plate 2. Haemoproteus from Philippine Birds

Figs. 19 to 25. *Haemoproteus* in the red-blood cells of the broad-billed roller, *Eurystomus orientalis. 19,* One small parasite; *20,* two larger parasites in one red cell; *21,* two parasites almost fully grown in one red cell; *22,* a fully grown and a young parasite in one red cell; *23,* two fully grown parasites in one red cell; *24,* three large parasites in one red cell; *25,* a parasite free in the blood stream.

Fig. 26. *Haemoproteus* in a red-blood cell of the Philippine sparrow hawk, *Accipiter manillensis.*

Figs. 27 to 31. *Haemoproteus* in the red-blood cells of the Java coucal, *Centropus javanicus. 27,* Uninfected red cell; *28,* parasite free in the blood stream; *29* to *31,* three fully grown parasites within red cells.

Figs. 32 to 34. *Haemoproteus* in the red-blood cells of the Philippine oriole, *Oriolus acrorhynchus.*

Fig. 35. *Haemoproteus* in a red-blood cell of Lindsay's kingfisher, *Halcyon lindsayi.*

All figures of Plate 2 were drawn with a camera lucida from specimens stained with Giemsa; they are all magnified 2,300 diameters.

PLATE 3. PROTOZOAN PARASITES OF PHILIPPINE ANIMALS

FIG. 36. *Balantidium* from a frog, *Rana vittigera.*
37. *Balantidium* from *Rana vittigera,* in division.
38. An *Endolimax* amœba from *Rana vittigera.*
FIGS. 39 to 41. *Plasmodium* from the Philippine bulbul, *Iole gularis. 39* and *40,* Parasites free in the blood stream; *41,* An uninfected red cell.
FIG. 42. A segmenting specimen of *Plasmodium* in a red-blood cell of the large-nosed shrike, *Cephalophoneus nasutus.*
FIGS. 43 to 46. *Plasmodium* in the red-blood cells of the gray-head shrike, *Otomela lucionensis. 43,* A parasite free in the blood stream; *44,* an uninfected red cell; *45* and *46,* fully grown parasites within red-blood cells.
FIG. 47. *Balantidium* from a frog, *Kaloula picta.*

All figures of Plate 3 were drawn with a camera lucida; figs. 36 to 38 and 47 from living specimens and the rest from specimens stained with Giemsa. Magnifications are as follows: Figs. 36 and 38, 1000 diameters; fig. 37, 250 diameters; figs. 39 to 46, 2,300 diameters; fig. 47, 500 diameters.

PLATE 4. PROTOZOAN PARASITES OF PHILIPPINE ANIMALS

FIG. 48. *Nyctotherus* from a cockroach, *Periplaneta americana.*
49. *Endamœba blattae* from a cockroach, *Periplaneta americana.*
50. *Endamœba* from the domestic fowl.
51. An amœba from a cockroach, *Periplaneta americana.*
52. *Endolimax* from the domestic fowl.
53. An amœba from a house rat.
54. *Lophomonas striata* from a cockroach, *Periplaneta americana.*
55. *Lophomonas blattarum* from a cockroach, *Periplaneta americana.*

All figures of Plate 4 were drawn with a camera lucida from living specimens. Magnifications are as follows: Fig. 48, 780 diameters; fig. 49, 500 diameters; figs. 50 and 52, 2000 diameters; figs. 51, 53, 54 and 55, 1000 diameters.

PLATE 5. FLAGELLATE PROTOZOAN PARASITES FROM HOUSEFLY AND PLANTS

FIG. 56. *Herpetomonas muscae-domesticae* from the intestine of a house-fly. Typical specimen at the right; dividing stages at the left.
57. *Phytomonas* from a plant, *Euphorbia hirta.* Typical specimen at the left; dividing stages at the right.

All figures of Plate 5 were drawn with a camera lucida, at a magnification of 2300 diameters, from specimens stained with Giemsa.

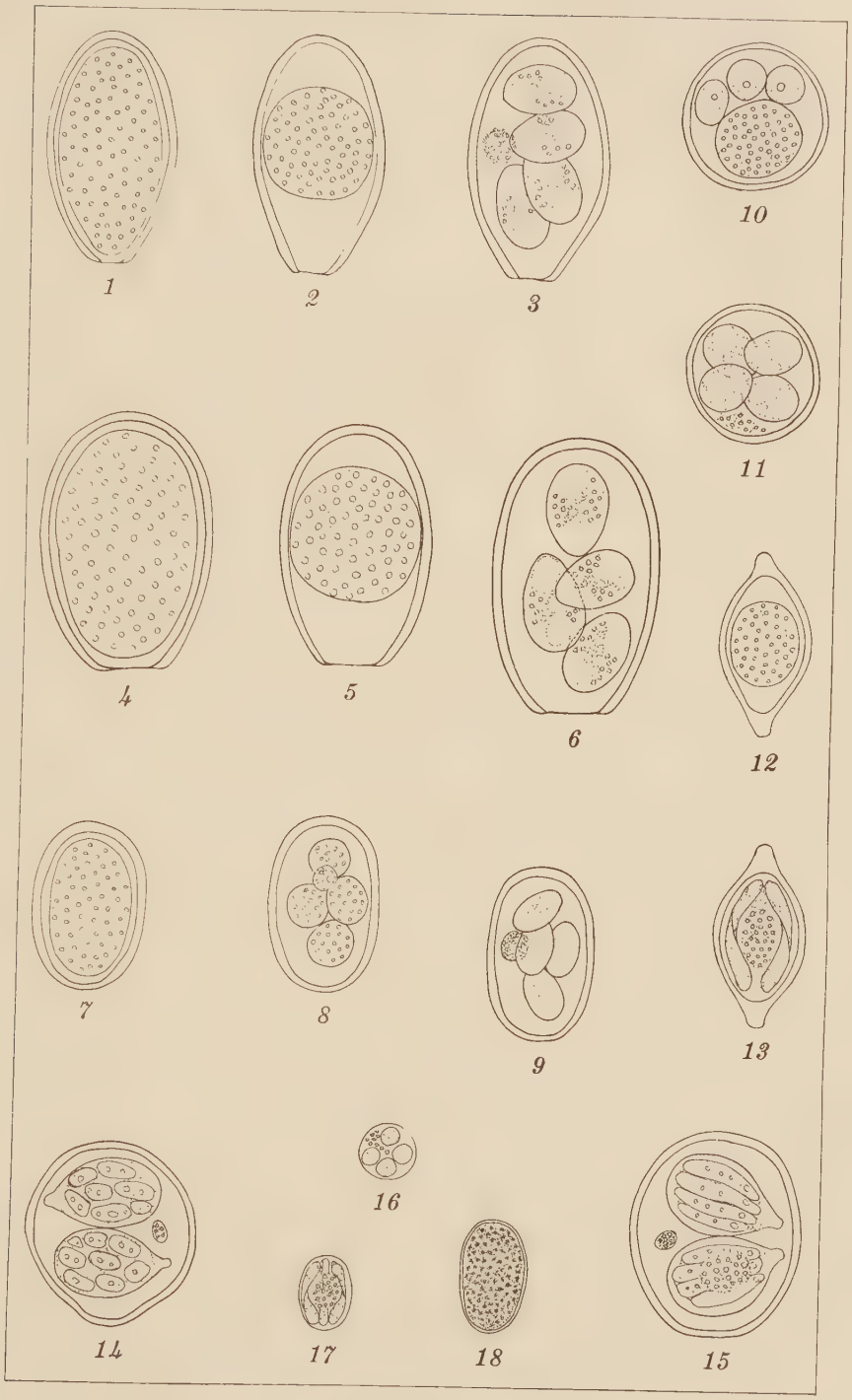


PLATE 1.

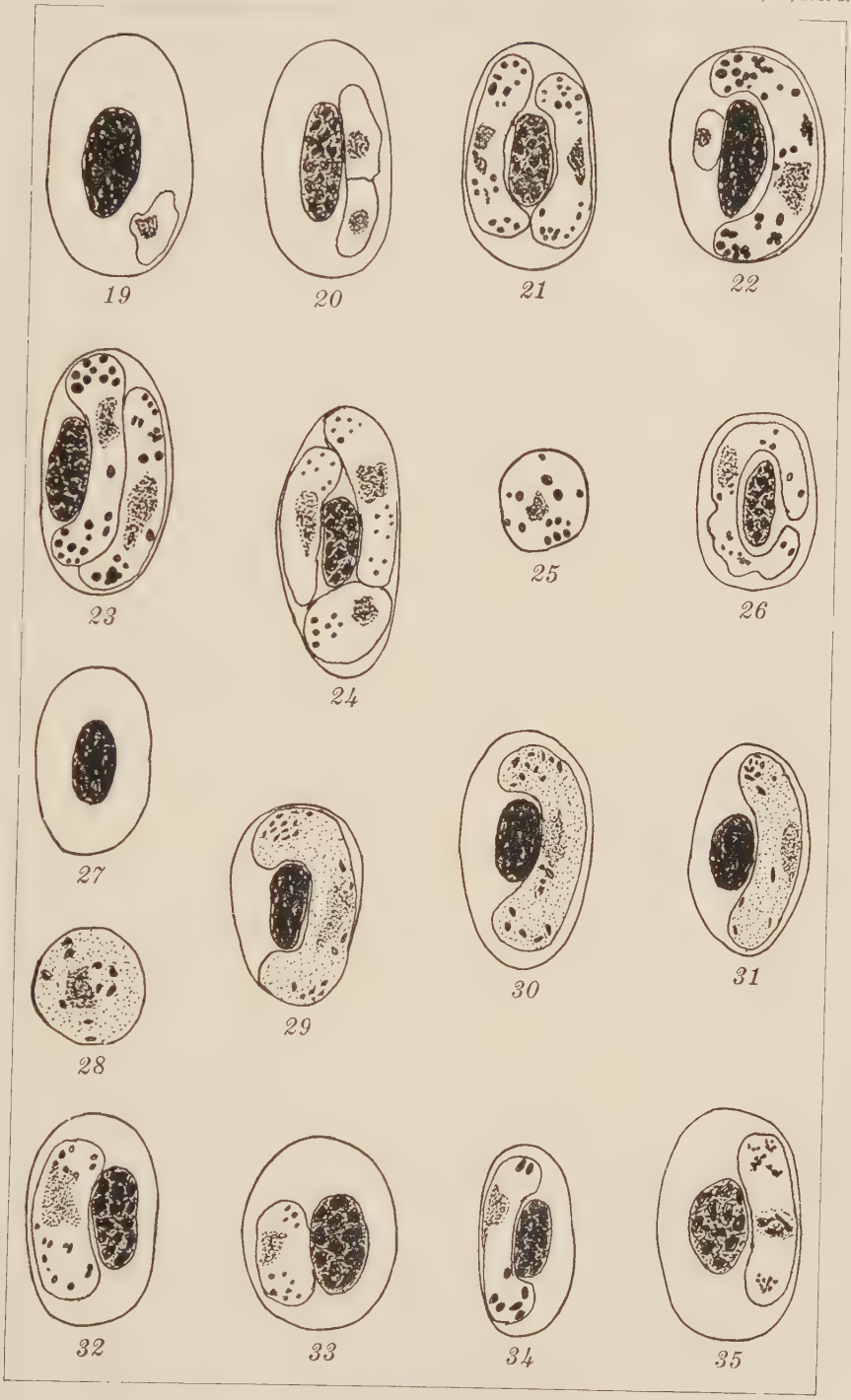


PLATE 2.

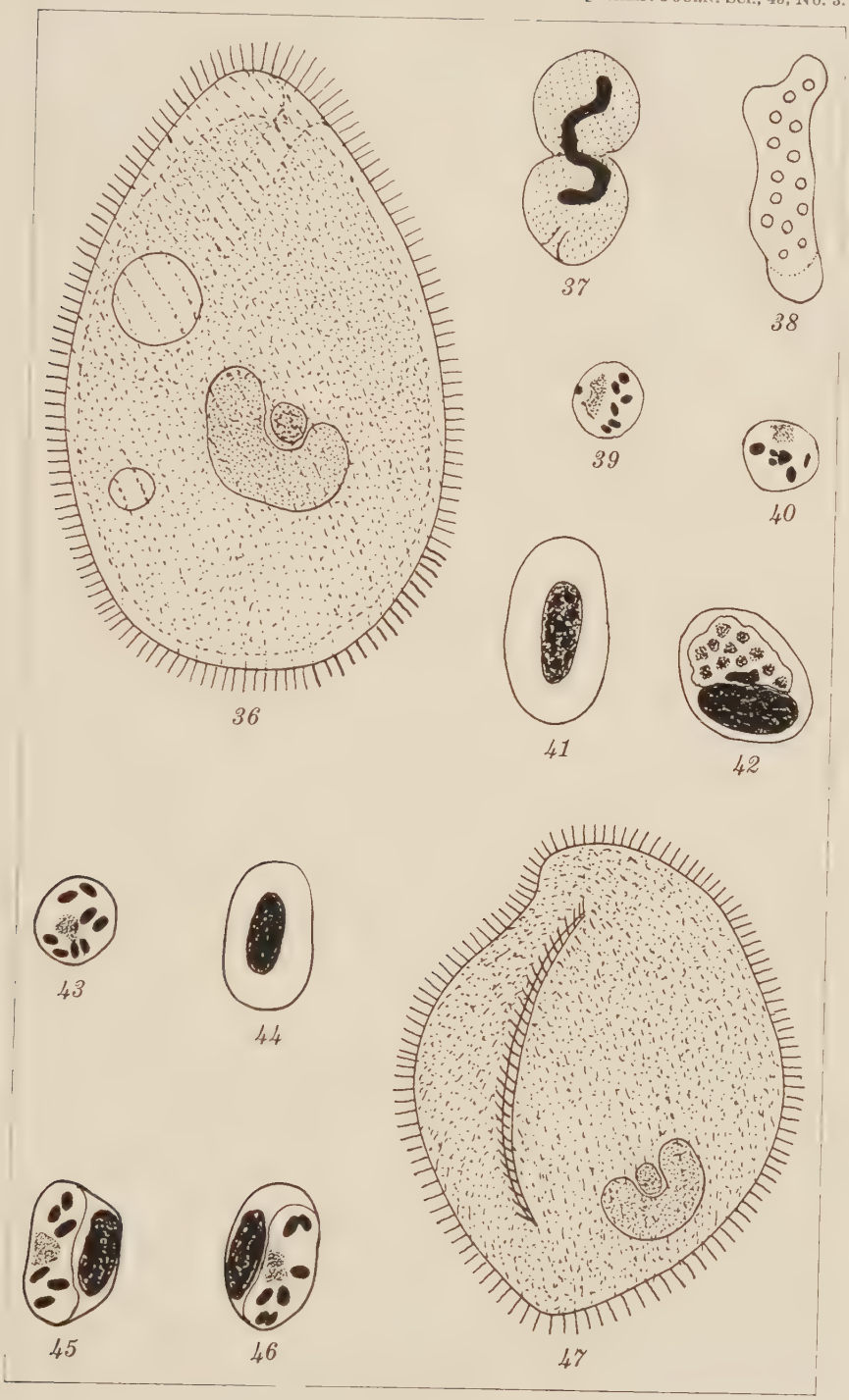


PLATE 3.

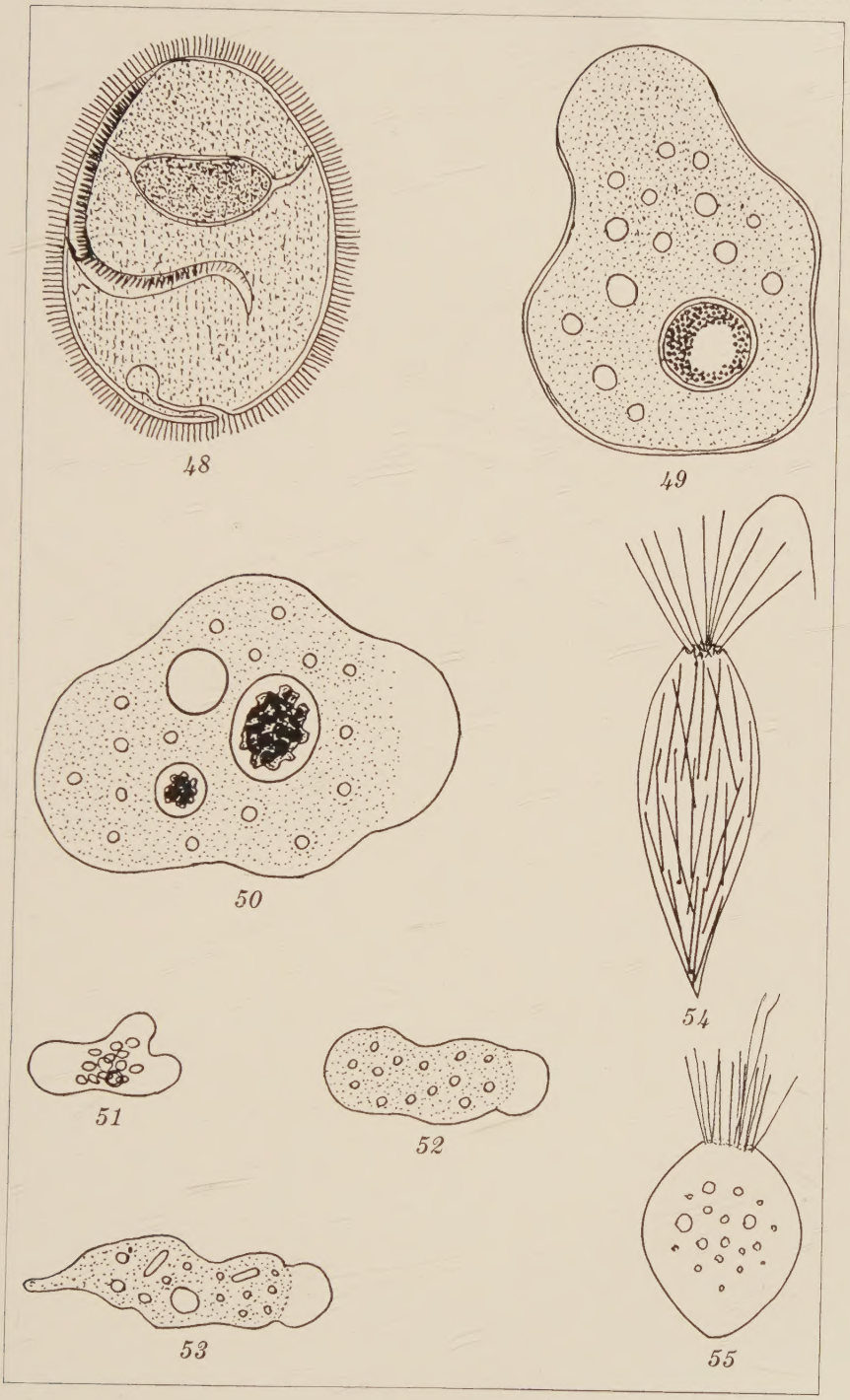


PLATE 4.

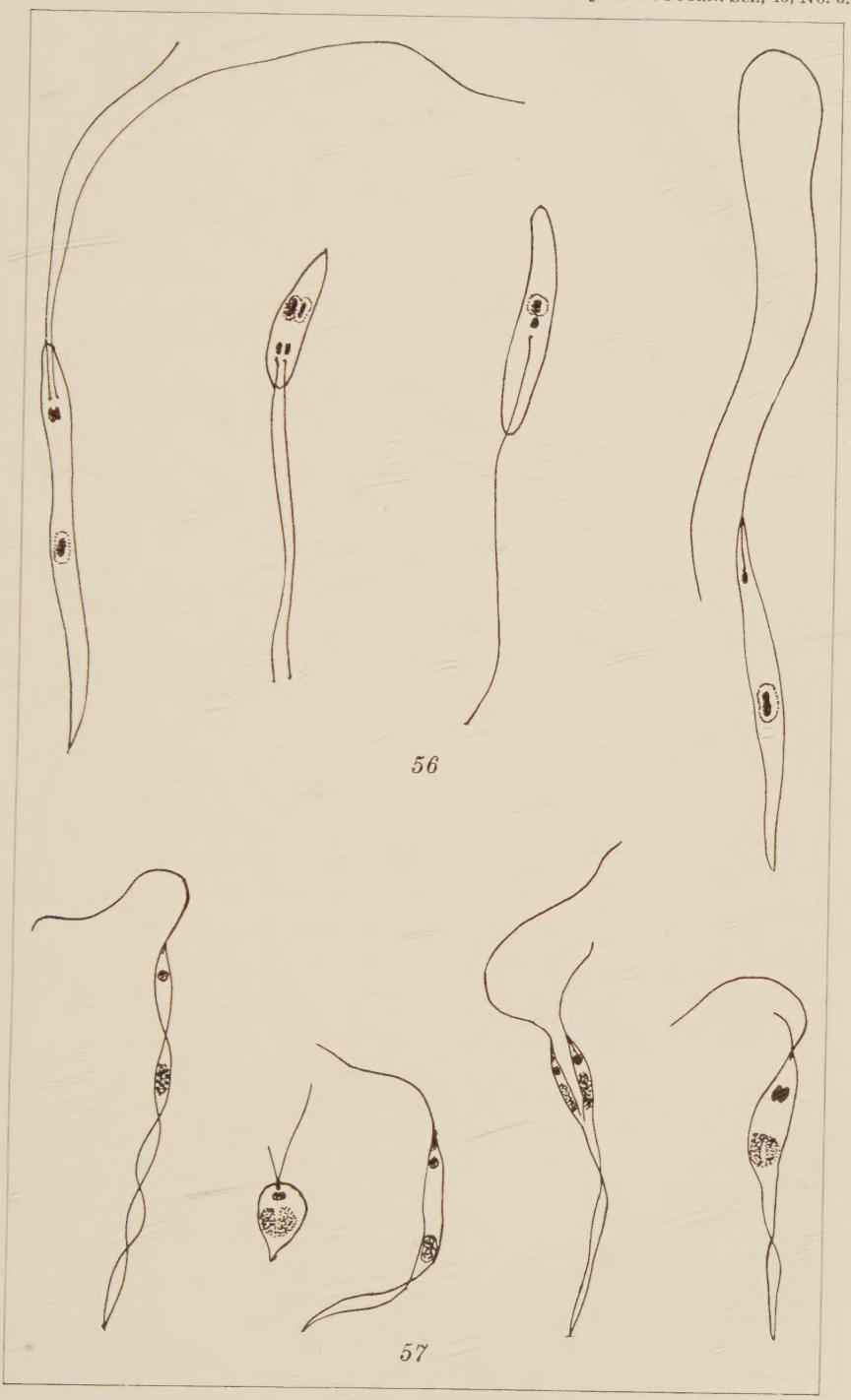


PLATE 5.